2021 Update Report

California's Cholinesterase Test Results Reporting and the Medical Supervision Program

Department of Pesticide Regulation
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Contributors:

Department of Pesticide Regulation (DPR)

Shafeesha Ali
Mia Cylinder
Lucia S. Graham, Ph.D., REHS
Yvette Nonato, MD, DPBRM
Michel Oriel

Office of Environmental Health Hazard Assessment (OEHHA)

Stephanie Hung, MS
Ouahiba Laribi, Ph.D., MPH

Reviewers:

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EXECUTIVE SUMMARY

The California Medical Supervision Program ("Program") is designed to protect agricultural workers who regularly handle Type I and II organophosphate (OP) and carbamate (CB) pesticides [Title 3, California Code of Regulations (CCR), section 6728]. It requires employers to contract with a medical supervisor to monitor the blood cholinesterase levels of their workers. Cholinesterase is critical for the normal function of the nervous system. Overexposure to OP and CB pesticides can lead to a depression in cholinesterase activity levels, which can lead to various adverse health effects. The California Department of Pesticide Regulation (DPR) is responsible for the overall administration of the Program. The Office of Environmental Health Hazard Assessment (OEHHA) is responsible for outreach and education of medical supervisors, and the California Department of Public Health (CDPH) is responsible for approving laboratories performing cholinesterase analysis.

The Program was established in 1974 when the use of cholinesterase-inhibiting pesticides was prevalent in California agriculture. DPR Pesticide Use Report data from 1995 to the present show the use of Type I and II OP/CB pesticides has declined by 89%. However, according to the most recent pesticide use data, Type I and II OP/CB use remained on average at approximately two million pounds per year from 2011 to 2019, thus highlights the need to continue to monitor and provide protection to workers who regularly handle these pesticides.

Assembly Bill (AB) 1963 (Statutes of 2010, Chapter 369) established the requirement for the reporting of laboratory cholinesterase test results to DPR. Reporting of cholinesterase test results is a series of steps that begins with the medical supervisor submitting a cholinesterase test order to the facility drawing the employee's blood. The blood drawing facility then transmits information on the test requisition slip to the laboratory performing the cholinesterase analysis, who then in turn sends the cholinesterase results and other required data elements to DPR. DPR and OEHHA, in consultation with CDPH, submitted a report to the Legislature in December 2015 evaluating the effectiveness of the Program. The cholinesterase test results included in the 2015 Report were collected from 2011–2013, and the departments determined that "overall the Program appears effective in protecting agricultural workers handling cholinesterase-inhibiting pesticides." However, the evaluation of the utility of laboratory-based reporting of cholinesterase test results was difficult due to certain challenges identified in the report. Recommendations for future directions to address these challenges were also included in the report.

Following up on the recommendations in the 2015 Report, AB 2892 (Statutes of 2016, Chapter 475) added new reporting requirements to improve the quality and quantity of the data being submitted, and extended the continued reporting of cholinesterase test results until January 1, 2021. (Subsequent legislation further extended the reporting requirements to January 1, 2023). From 2014–2019, DPR received over 140,000 cholinesterase test results from the reporting laboratories. As with previous years, a majority of the reported tests appeared to have been ordered for clinical reasons unrelated to the Program. In this report, some improvements to the data cleaning and analysis methodology led to better identification of individuals undergoing cholinesterase testing under the Program. In addition to evaluating the pattern of cholinesterase test results, other efforts conducted by DPR and OEHHA added to the departments' knowledge of the overall effectiveness of the Program. These efforts included the inspection of pest control businesses in high-use OP/CB areas, recent changes to Health and Safety Code § 105206, and outreach to medical supervisors and employers. The following provides findings and recommendations based on the current analysis.

Summary of Findings

Overall, similar to conclusions found in the 2015 Report, the Program appears effective in protecting agricultural workers handling cholinesterase-inhibiting pesticides. The utility of laboratory-based reporting of cholinesterase test results from 2014 to 2019 was evaluated and results of this evaluation were similar to those presented in the 2015 Report. Although there were some improvements in the data quality observed since 2014, the utility of the data analysis continues to be hampered by the inclusion of thousands of records from individuals who are not in the Program, and by missing data on the purpose of the test. Despite difficulties in obtaining complete information, DPR and OEHHA were able to identify individuals as part of the Program and to estimate cholinesterase depressions.

The analysis of the cholinesterase data indicates that most individuals identified as part of the Program did not have significantly depressed cholinesterase activity levels. Through this analysis DPR and OEHHA were also able to identify some individuals whose cholinesterase activity was depressed enough to necessitate their removal from the workplace, thereby protecting these workers from further exposures. Moreover, most of the physicians who regularly ordered cholinesterase tests were medical supervisors, a marked improvement from 2014. However, due to the frequency of the submission of the cholinesterase test reports by the laboratory and level of processing required of the data, analysis and real-time detection of individuals with depressed cholinesterase activity levels are not currently feasible.

Previous surveys conducted by DPR and OEHHA to Program participants showed varying levels of understanding of specific requirements. Outreach efforts since the last report have resulted in improvements of participants' understanding of the Program, and in the quality of the cholinesterase reports received. Additionally, the cholinesterase test results reports have been useful in identifying which ordering physicians are medical supervisors in order to inform them about the Program's registration process, and conduct targeted outreach and training. Further enhancement of educational materials and outreach efforts to improve communications among all Program participants would strengthen efforts to monitor the Program's effectiveness and enhance protection of California's agricultural workers.

Since AB 2892 (Statutes of 2016, Chapter 475) was adopted, DPR has not received any pesticide illness reports due to cholinesterase depression from local health officers, and the reason for this is not known, albeit 12 test results, from a total of five individuals, with the term "recovery" indicated as purpose of test were identified among the ChE test results ordered by medical supervisors. OEHHA and DPR are following up with medical supervisors, and will gather more information that could help determine why none of these cholinesterase depressions were reported as pesticide illnesses.

Future Directions

Although the recommendations proposed in the 2015 Report were carried out and provided some useful information, similar shortcomings were identified in this update report. Along with current ongoing activities, DPR and OEHHA plan to take the following steps to help enhance the Program's effectiveness and utility of laboratory-based reporting:

DPR/OEHHA – Future Directions	Leads/ *Participants*	Requires Legislation?
• Focus the next evaluation on counties with high OP/CB use and conduct monitoring study. *Rationale: to evaluate the components of the Program on a smaller scale to better assess its effectiveness*.	Leads: DPR, OEHHA	No
• Amend CCR Title 3 § 6728(c)(1) to add the recommended time frame for performing ChE baseline testing for workers under the Program to be consistent with OEHHA's *Guidelines for Physicians*. *Rationale: to align the requirements for employers with OEHHA's Guidelines for Physicians.*	Lead: DPR	No
• Amend HSC § 105206 to request additional data elements from reporting laboratories to better identify workers and ordering physicians. *Rationale: to help better identify individuals under the Program.*	Leads: DPR, OEHHA	Yes

BACKGROUND AND INTRODUCTION

The California Medical Supervision Program ("Program") is designed to protect agricultural workers who regularly handle organophosphate and carbamate pesticides (OP/CB) [authorized by Food and Agricultural Code section 12981, and implemented by Title 3, California Code of Regulations (CCR), section 6728; Appendix A1]. The Program requires employers to contract with a licensed physician as a "medical supervisor"[1] to monitor the blood cholinesterase (ChE) levels of their workers. The enzyme ChE is critical for the normal function of the nervous system. Overexposure to OP and CB pesticides can lead to a depression in ChE activity levels, which can lead to various adverse health effects (Appendix D1). The California Department of Pesticide Regulation (DPR) is responsible for the overall administration of the Program. The Office of Environmental Health Hazard Assessment (OEHHA) is responsible for outreach and education of medical supervisors, and the California Department of Public Health (CDPH) is responsible for approving laboratories performing ChE analysis.

The Program was established in 1974 when the use of ChE-inhibiting pesticides was prevalent in California agriculture. Pesticide Use Report (PUR) data from 1995 to the present shows the agricultural-use of Type I and II OP/CB pesticides has declined significantly (89%), averaging two million pounds per year from 2011 to 2019 (Figure 1).

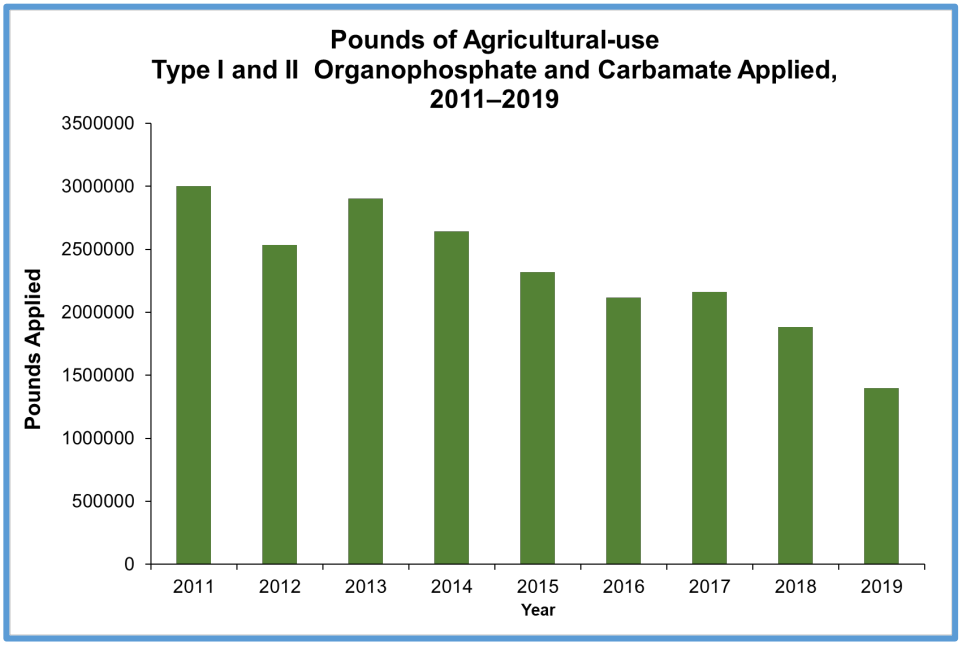


Figure 1: *Reported pounds of agricultural-use Type I and Type II OP and CB pesticides applied in California, 2011–2019.*

[1] Under HSC § 105206, a medical supervisor is a licensed physician (M.D. or D.O.) who has a written agreement with employers of agricultural workers who regularly apply cholinesterase-inhibiting pesticides in Toxicity Categories I and II, to examine the employees for fitness, order cholinesterase tests, and to make the necessary recommendations based on the results of an employee's cholinesterase test results (Appendix A2).

Assembly Bill (AB) 1963[2] (Statutes of 2010, Chapter 369) added a laboratory-based reporting requirement (Appendix A2) to evaluate the Program. Medical supervisors were required to indicate the "purpose" of the ChE test on the laboratory test requisition slip. Additionally, laboratories that perform ChE analysis on human blood drawn in California as part of the Program were required to report to DPR the test results, purpose of the test, specific information pertaining to the employee, his/her employer, the medical supervisor and the laboratory performing the analysis.

The framework for the ChE test results reporting involves a series of data transfers from the employer/employee to the medical supervisor to the blood-drawing facility to the reference or reporting laboratory to DPR:

- The medical supervisor orders a ChE test for the employee and provides information that should include employment information and the purpose of the ChE test to the blood-drawing facility.
- The blood-drawing facility may or may not have the capability to capture the information provided by the medical supervisor or to transmit the information to the laboratory performing the ChE analysis.
- The reporting laboratory electronically submits the ChE test results, along with the information provided by the blood-drawing facility, to DPR.

The accurate transfer of all data elements under Health and Safety Code (HSC) section 105206 requires each party to collect and submit the information to the next party in the data chain. The quality of the data received by DPR is entirely dependent on successful submission by the ordering physician, and the ability of the laboratories to capture and transfer all of the required data elements.

In December 2015, in accordance with HSC § 105206, a report evaluating the effectiveness of the Program and the utility of the laboratory-based reporting of ChE test results for pesticide-related illness surveillance and prevention was submitted to the Legislature[3]. The report was a collaborative effort between DPR and OEHHA, in consultation with CDPH. ChE test results submitted to DPR by the laboratories from 2011–2013 were included in the report. In addition, supplementary activities were conducted to better evaluate the Program, such as 1) conducting a mail survey of physicians who ordered ChE tests, 2) conducting in-person visits with medical supervisors, and 3) inspecting employment records of a select group of employers in areas of high OP/CB use. The report concluded that "overall the Program appears to be effective in protecting agricultural workers handling cholinesterase-inhibiting pesticides." However, the evaluation of the utility of the laboratory-based reporting of ChE test results (2011–2013) was difficult due to the challenges identified in the report, such as laboratories reporting ChE test results regardless of the relevance to the Program, missing data on the purpose of the test, and an inability to identify physicians ordering ChE tests as medical supervisors. The report also included recommendations for future direction to address the challenges identified (Appendix A3).

[2] Codified into law as Health and Safety Code section 105206 that took effect on January 1, 2011.
[3] DPR and OEHHA, Report to the California Legislature: California's Cholinesterase Test Results Reporting and the Medical Supervision Program, California Environmental Protection Agency, December 2015. Available at https://oehha.ca.gov/media/downloads/pesticides/report/reporttolegislature2015.pdf.

Acting on the recommendations in the 2015 Report, the Legislature passed AB 2892 (Statutes of 2016, Chapter 475) to amend HSC § 105206, requiring:

- Employers to contract only with physicians registered with OEHHA as medical supervisors.
- Changes in terminology for "purpose" of ChE test to be provided by the medical supervisor, consistent with that in OEHHA's *Guidelines for Physicians Who Supervise Workers Exposed to Cholinesterase-inhibiting Pesticides* (*Guidelines for Physicians*).
- Medical supervisors to report any worker with ChE depression indicating pesticide exposure to the local health officer pursuant to HSC § 105200.
- Continued reporting of ChE test results to DPR until January 1, 2021. In 2020, the Legislature passed Assembly Bill 3220, which extended the reporting of ChE test results to DPR until January 1, 2023.

In accordance with HSC § 105206(g), this 2021 report evaluates the effectiveness of the Program and utility of laboratory-based reporting of ChE test results for illness surveillance and prevention. This report lays out the actions taken since AB 2892 (Statutes of 2016, Chapter 475) (effective January 2017) and an update to the analysis of the ChE test results.

This report is a collaborative effort between DPR and OEHHA.

ACTIONS TAKEN AND CHANGES MADE SINCE THE 2015 REPORT

Following the recommendations from the 2015 Report, OEHHA and DPR have taken a series of actions in an attempt to improve the data quality of the ChE test results submitted by the reporting laboratories and/or the Program itself.

Registration of Medical Supervisors (OEHHA)

Since AB 2892 (Statutes of 2016, Chapter 475) was enacted, OEHHA has developed a registration process[4] and in 2018 adopted that process in regulation (17 CCR § 98201 et seq.). OEHHA annually registers physicians as medical supervisors pursuant to this process.

- A registration form developed by OEHHA is available online for physicians to download (https://oehha.ca.gov/media/downloads/pesticides/document/medsuperegforma5.pdf).

To identify potential medical supervisors under the Program, OEHHA uses ChE test results submitted by the laboratories from previous years and informs all physicians who ordered at least 10 ChE tests about the new mandatory registration.

A list of currently registered physicians is posted on OEHHA's website with a map to help employers identify medical supervisors by proximity (Figure 2). This list is continuously updated when physicians register into and de-register from the Program.

[4] Health and Safety Code § 105206(f): The OEHHA shall establish a procedure for registering and deregistering medical supervisors for the purposes of outreach and training and may establish reasonable requirements for performance.

- As of October 6, 2020, OEHHA has registered 105 physicians as medical supervisors (https://oehha.ca.gov/pesticides/general-info/list-registered-medical-supervisors).

OEHHA contacts registered physicians on an annual basis to inform them of the need to renew their registration and to report any ChE test result depression indicative of pesticide exposure.

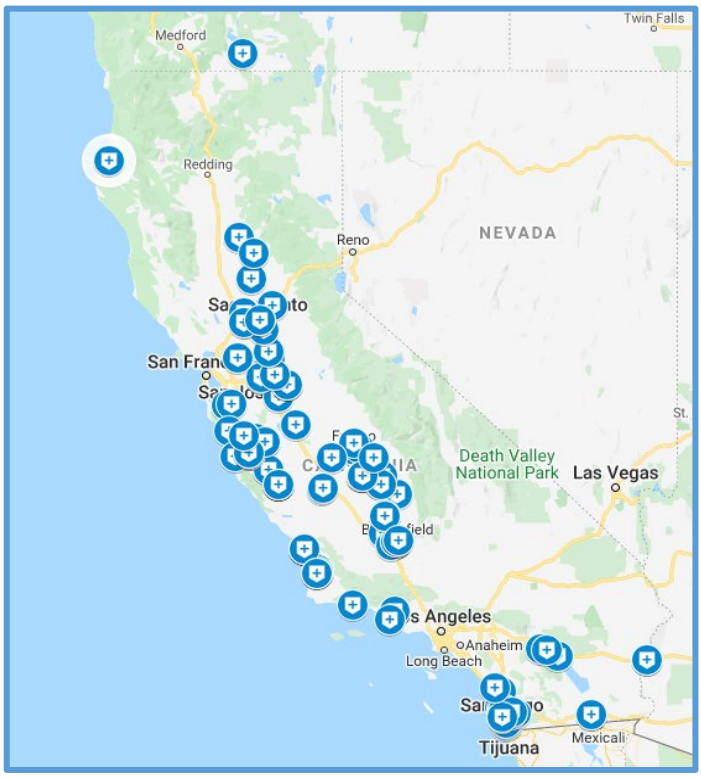


Figure 2: *Geographic distribution of registered medical supervisors.*

Outreach to Registered Medical Supervisors (OEHHA)

OEHHA communicated with registered physicians to share new or updated materials. Links to these materials are sent to physicians at the time of registration.

- In 2017, OEHHA updated its *Guidelines for Physicians* and its training course to reflect changes to HSC § 105206 (https://oehha.ca.gov/pesticides/general-info/medical-supervisor-guidelines).
- A flow chart and a calculator was introduced on OEHHA's website in 2019 to guide medical supervisors with their monitoring of ChE activity levels for handlers under the Program (https://oehha.ca.gov/pesticides/general-info/medical-supervisor-calculator).
- Additionally, in 2020, OEHHA created a 10-minute training video on the Program so physicians and other health care professionals interested in the Program can quickly learn the basics and the main responsibilities of medical supervisors (https://oehha.ca.gov/pesticides/california-medical-supervision-program).
- OEHHA continues to offer in-person trainings on the Program and gave trainings in five different locations since the 2015 Report.

Outreach to Employers, Handlers of OPs/CBs, and CACs (DPR)

DPR developed outreach materials for employers, OP/CB handlers, and County Agricultural Commissioners (CACs).

- In 2018, DPR updated the Pesticide Safety Information Series handout on Extra Medical Care for Handlers Who Use OPs/CBs to reflect changes to the Pesticide Worker Safety regulation in Title 3, CCR (Food and Agriculture, Subchapter 3 on Pesticide Worker Safety). This informational material is used in DPR's continued outreach efforts and trainings to employers. (Appendix B1).
- DPR prepared factsheets on the requirements of 3 CCR § 6728 specifically for employers who have employees who are regular handlers of OPs/CBs.
  - These materials were distributed to employers and Pest Control Businesses (PCBs) through the CACs, when they come to the county office for permits to apply OPs and CBs (Appendix B2). Permits are issued annually with peak permit season being in January–February and into March or later for some crops.
  - Another handout developed by DPR in 2018 was a Frequently Asked Questions (FAQ) handout targeted for employers (Appendix B3). This material summarizes the essential requirements of the Program and is primarily used as a training aid for employers and their employees who are regular handlers of OPs/CBs.
- DPR also distributes these outreach materials and information about the Program at agricultural events, trainings, and conferences.

Outreach to Laboratories (DPR with CDPH)

In 2016, DPR coordinated with CDPH on outreach efforts to the laboratories in order to improve the quality of reporting required in HSC § 105206. As a result, CDPH updated the application forms used by laboratories seeking approval to perform ChE analysis for occupational health surveillance.

- Since laboratories were able to customize specific entries on the "purpose of test" into their requisition slip or electronic ordering portals based on the physician's request, CDPH did not address the addition of the purpose of test on requisition slips with the laboratories.
- CDPH updated their website to reflect the laboratories approved to perform ChE tests under the Program. As of April 2019, there are five reporting laboratories (https://www.cdph.ca.gov/Programs/CCDPHP/DEODC/EHLB/Pages/CDPH-Approved-Cholinesterase-Laboratories.aspx).

Pest Control Businesses Inspection and Survey Project (DPR)

One of the recommendations in the 2015 Report was to conduct a survey and inspection of agricultural Pest Control Businesses (PCBs). This project is similar to the Focused Growers' Headquarters Inspection project (See Appendix F in the 2015 Report) that was completed in 2014. In 2017, DPR launched an inspection and survey

of PCBs that showed the highest use of OP/CB pesticides based on PUR, and located in counties that showed the highest reports of ChE tests.

- The objective of this project was to determine PCBs' knowledge of the Program, and their compliance with its specific requirements.
  - DPR created a survey questionnaire to capture each of the requirements of 3 CCR § 6728, and an employee table to list regular handlers of selected PCBs (Appendices C2 and C3).
  - 50 PCBs in areas of high OP/CB use (distributed around the state) were identified to be inspected/surveyed.
- This project was completed in 2018. See Appendix C for protocol and survey forms.

Pesticide Illness Reporting

AB 2892 (Statutes of 2016, Chapter 475) required medical supervisors to report ChE depression indicating pesticide exposure to the local health officer as of January 1, 2017. Since AB 2892 was adopted (2017), DPR has not received any pesticide illness reports due to a ChE depression from local health officers, albeit 12 test results, from a total of five individuals, with the term "recovery" indicated as purpose of test were identified among the ChE test results ordered by medical supervisors. Because the law does not specify the level of ChE depression that requires reporting, OEHHA conducted literature reviews to identify a threshold for reporting.

- In order to assess which level of ChE depression indicates pesticide exposure, OEHHA performed a systematic literature review to identity epidemiological studies linking exposure to OPs and/or CBs and ChE activity levels (Appendix D2). While studies showed that exposure to OPs and CBs induce inhibition of ChE activity levels in both red blood cell (RBC) and plasma, due to the variability in study design and toxicity and amount of pesticides used, OEHHA could not define with certainty a specific threshold level of depression that would indicate exposure to these pesticides.
- In order to identify variations of ChE activity levels without pesticide exposure, OEHHA performed a literature review of intra- and inter-individual ChE variations in the population of working adults with no known exposures to ChE-inhibiting chemicals (Appendix D3). OEHHA found that while inter-individual variation can be significant, variation of ChE within the same individuals is much smaller for both plasma and RBC ChE. OEHHA also examined intra-individual variation in baseline estimates from the individuals in the Program and found similar results (see Appendix G, supplemental study).
- The reason(s) behind the lack of reporting is not entirely clear at this time. OEHHA and DPR suggest to investigate this issue by coordinating activities such as surveying medical supervisors, exploring the possibility that ChE test results of workers under the Program be submitted by medical supervisors or employers directly to DPR, and also, exploring the possibility of utilizing a sole state-run laboratory. OEHHA and DPR would then recommend actions to address this problem, such as proposing regulation(s) or expanding outreach to physicians. The results of the various literature reviews will be used to further evaluate these recommendations.

FINDINGS

Sources of ChE Test Results

For the most part, the ChE test results received by DPR in 2014–2019 were reported by the same six laboratories that reported in 2011–2013. In 2016, a new laboratory was added to the CDPH list of laboratories approved for ChE testing, LABCORP. This laboratory began reporting in March 2017 (Table 1). Two laboratories, PALI and MEDTOX, discontinued ChE testing in 2018 and 2019, respectively. As of April 2019, there are five laboratories approved for ChE testing (Appendix E).

Table 1: *Laboratories that perform ChE Test Analysis, as of April 2019.*

Laboratories	Acronym
ARUP Laboratories	ARUP
Laboratory Corporation of America	LABCORP
Pacific Toxicology Laboratories	PACTOX
Quest Diagnostics Laboratory, Sacramento	QDI-SAC
Quest Diagnostics Laboratory, San Juan Capistrano	QDI-SJC

ChE Test Results Received from Laboratories

As with prior years, laboratories are still not able to distinguish ChE tests ordered under the Program from those that are performed for other reasons. Therefore, DPR continues to receive ChE test results from blood specimens drawn throughout the state, regardless of whether or not they are collected for the Program. The ChE reports submitted to DPR include results from pre-operative testing, Alzheimer's drug monitoring, liver disease screening, and occupational monitoring not under the Program (e.g., HAZMAT). Furthermore, DPR continues to manually review the data to: identify and remove duplicates, correct formatting errors, identify missing information, and correct typographical errors (Appendix F). DPR is also proactively working with reporting laboratories to improve data quality (e.g., data entry errors, discrepancies, etc.).

- The total number of ChE test results reported by the laboratories from 2011–2013 was 89,381, stemming from 42,189 test orders (Figure 3).
- From 2014–2019, the total number of ChE test results reported by the laboratories was 148,057, stemming from 70,510 test orders.
- The number of ChE test results and orders submitted by the reporting laboratories per year has been steadily decreasing since 2016, on average 9% per year, with 2019 being the lowest since the mandatory reporting requirement commenced. This correlates with the decrease in Type I and II OP/CB use.
- ChE test results from QDI-SAC represented a majority (79.1%) of the ChE test results received in 2019. This increase was due to a client or drawing laboratory transitioning from QDI-SJC to QDI-SAC as the reporting laboratory.

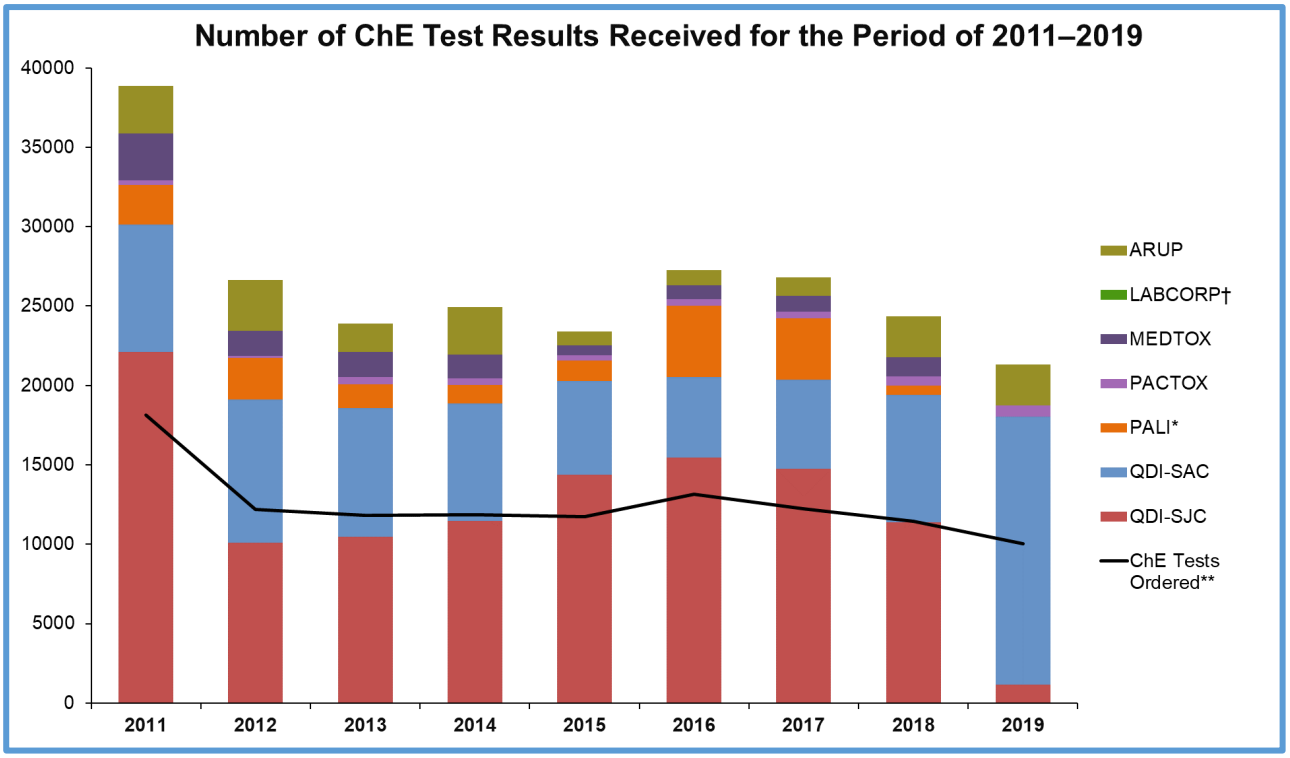


Figure 3: *Yearly number of ChE test results received by DPR from the reporting laboratories between 2011 and 2019. † -LABCORP submitted 20 ChE test results from 2017–2019. * -The increase in ChE test results from PALI in 2016 and 2017 was due to the laboratory submitting 5 different assay results, instead of 2, per specimen sample. ** -Number of ChE tests ordered by health care providers as reported by the laboratories.*

Purpose of Test from ChE Reports

HSC § 105206(c) was updated to include the use of specific terminology for purpose of test consistent with OEHHA's *Guidelines for Physicians* (see Glossary of Terms). Although the purpose of the ChE test is a data element required under HSC § 105206, a large proportion of the ChE test results reported by the laboratories to DPR do not include this information. The laboratories reported that this information was often left out either because the purpose of test was not indicated by the ordering physicians, the purpose of test was not transmitted to the laboratory, or the ChE test was not for an individual in the Program. Furthermore, even when the purpose of test was provided on the ChE reports, alternative terminology was used (Table 2).

Table 2: *Purpose of test terminology and terms submitted on the ChE reports.*

Purpose of Test	Alternative Terms Used in ChE Reports
Baseline	Baseline 1, Baseline 2, New Hire, Pre-employment, BL#1, Variants with typographical errors
Follow-up	Routine, Monitoring, Periodic Testing, Surveillance
Recovery	2nd Recovery, Recovery Draw
Suspected Illness	Pesticide Exposure, ChE Exposure, Chemical Exposure, Possible Exposure

As discussed in the 2015 Report, several laboratories were able to modify their requisition

form based on a physician request or have made changes to their online test order interfaces. There was a slight increase in the number of ChE test results with purpose of test indicated from 2014–2019 (Figure 4).

- From 2011–2013, 10,430 (11.7%) of ChE test results submitted by the laboratories indicated the purpose of test using terms related to the Program as described in Table 2.
- From 2014–2019, 27,329 (18.5%) of ChE test results submitted by the laboratories indicated the purpose of test using terms related to the Program as described in Table 2.

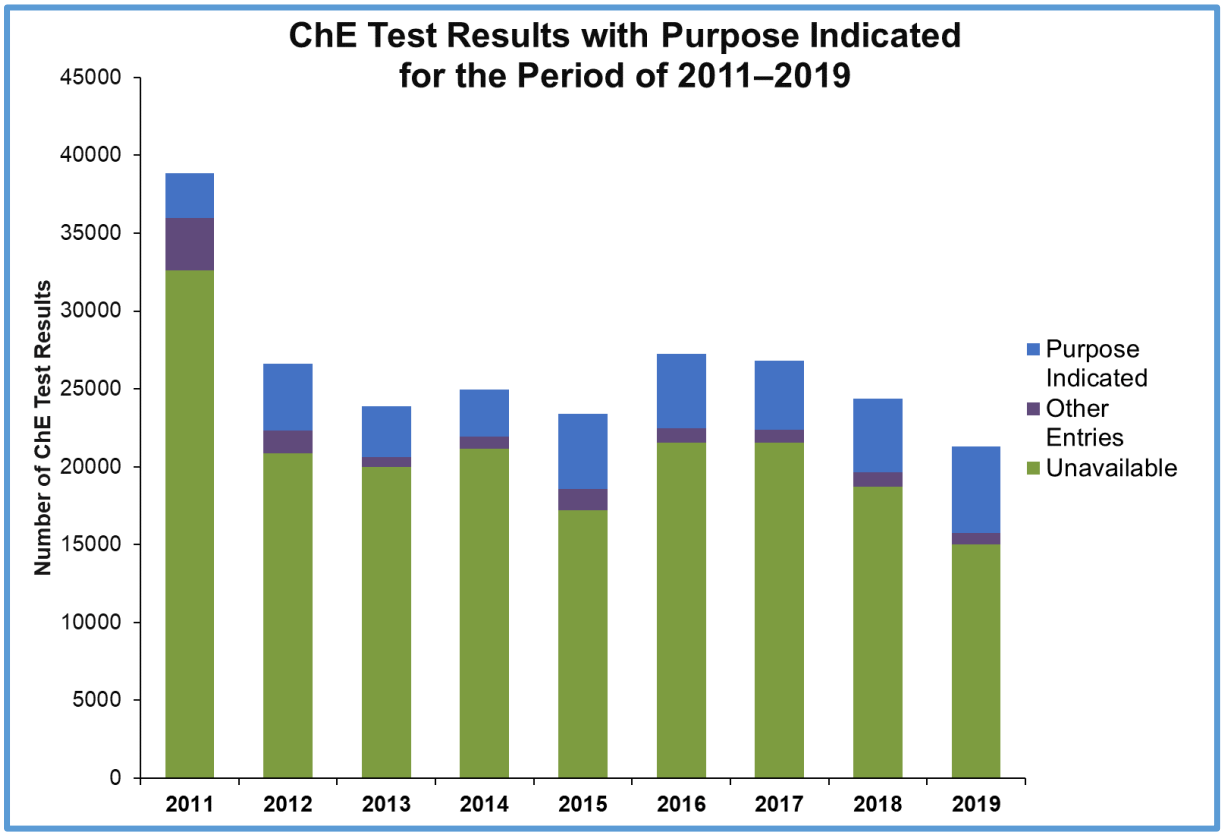


Figure 4: *Number of ChE test results with purpose of test as indicated in the reports submitted by the laboratories between 2011 and 2019.*

Even when the purpose of test is indicated, it cannot be determined definitively if the ChE test results received were related to the Program. Therefore, in addition to purpose of test, medical supervisor and employer information are needed to determine if the ChE test results were related to the Program.

ChE Tests Ordered by Physicians Reporting under the Medical Supervision Program

A total of 117 medical supervisors, identified through either DPR's Medical Supervisor Survey (2014), OEHHA's Medical Supervisor Outreach project (2015), or registered with OEHHA since 2017, submitted ChE test orders from 2011–2019 (Figure 5).

- From 2011–2013, medical supervisors submitted 11,921 (28.3%) test orders[5] for ChE analysis to the laboratories. Other health care providers, including those we were not able to identify as medical supervisors, submitted 30,268 (71.7%) ChE test orders, of which 18,570 (61.4%) test orders were missing provider information.
- From 2014–2019, 33,351 (47.3%) test orders were submitted by medical supervisors to the laboratories for ChE analysis. Other health care providers submitted 37,159 (52.7%) ChE test orders, of which 28,318 (76.2%) test orders were missing provider information.
- There has been a significant increase in the number of ChE test orders submitted by medical supervisors in the last few years. This might be due to DPR's Medical Supervisor Survey and OEHHA's outreach efforts.

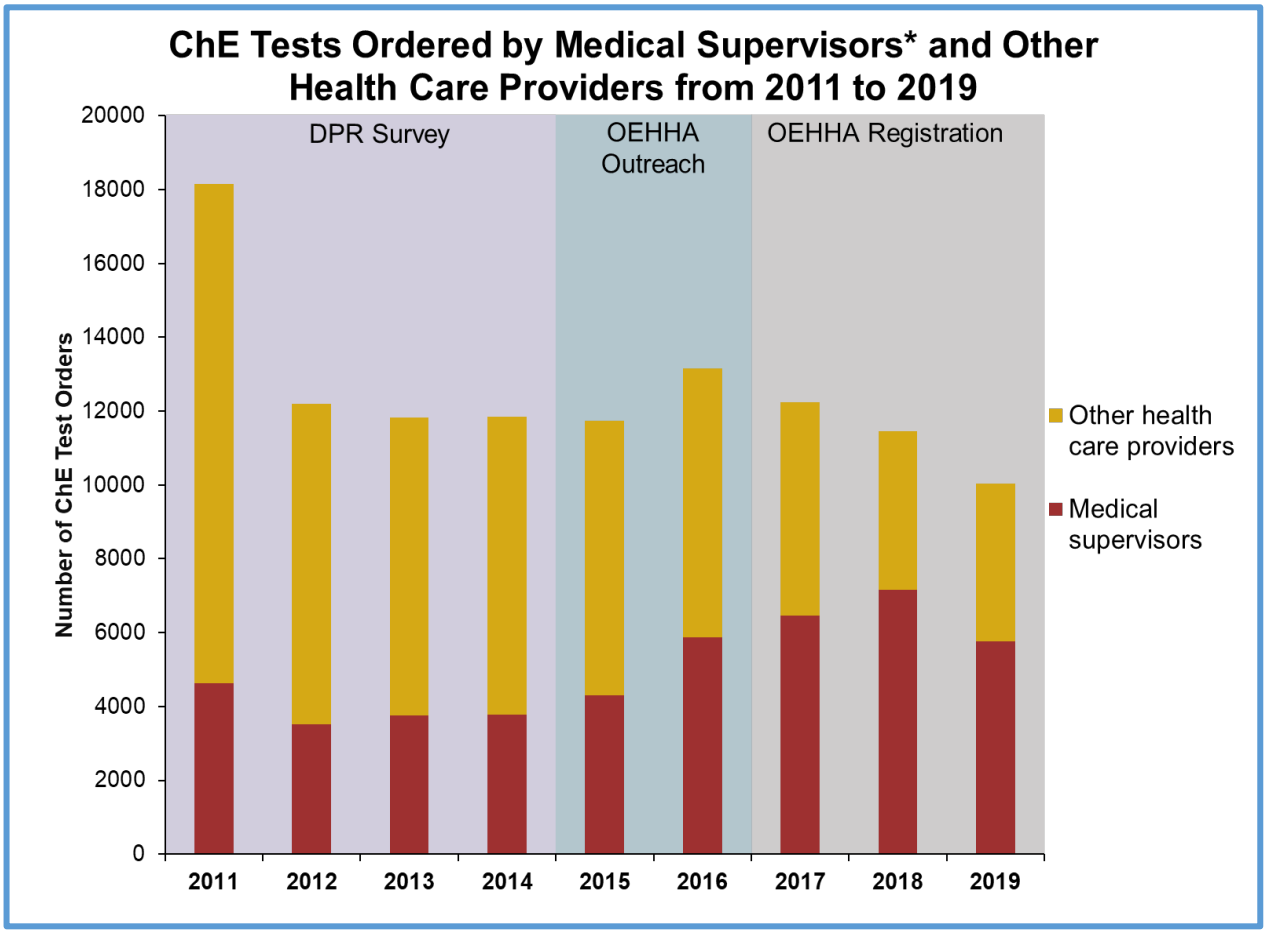


Figure 5: *Number of ChE tests ordered by medical supervisors and other health care providers as indicated in the reports submitted by the laboratories between 2011 and 2019. DPR identified medical supervisors in a survey from 2011 to 2014. OEHHA conducted outreach to medical supervisors from 2015 to 2016. OEHHA began registering medical supervisors in 2017. * -Physicians were identified as medical supervisors by DPR in 2014, by OEHHA's outreach project in 2015, or they were registered as medical supervisors with OEHHA (as of October 6, 2020).*

[5] A single ChE test order submitted to the laboratory generates two test results, RBC and plasma ChE. Reporting laboratories may include other ChE results in addition to the standard RBC and plasma ChE test results, e.g., RBC ratio to Hb or pseudocholinesterase.

Although an assumption can be made that ChE tests ordered by physicians on OEHHA's registry or confirmed by DPR are for employees under the Program, it is important to note that medical supervisors often work in occupational clinics and may order ChE tests for other employment purposes (e.g., HAZMAT). Therefore, other data elements such as the purpose of test (using terms described in Table 2) and employer were used to identify with confidence that ChE tests ordered were under the Program (Figure 6).

- From 2011–2013, 11,236 (94.3%) of ChE test orders submitted by medical supervisors to the laboratories were for known or likely agricultural employees (e.g., employees of growers or agricultural PCBs), of which 2,065 (18.3%) of ChE tests ordered indicated the purpose of test as reported by the laboratories.
- From 2014–2019, 30,757 (92.2%) of ChE test orders submitted by medical supervisors to the laboratories were for known or likely agricultural employees, of which 9,594 (31.2%) ChE tests ordered by medical supervisors with purpose of test as reported by the laboratories.
- Of the ChE tests ordered by medical supervisors that did not indicate purpose of test using terms related to the Program, 9,171 (81.6%) were for known or likely agricultural employees from 2011–2013, and 21,163 (68.8%) ChE test orders from 2014–2019 were for known or likely agricultural employees.

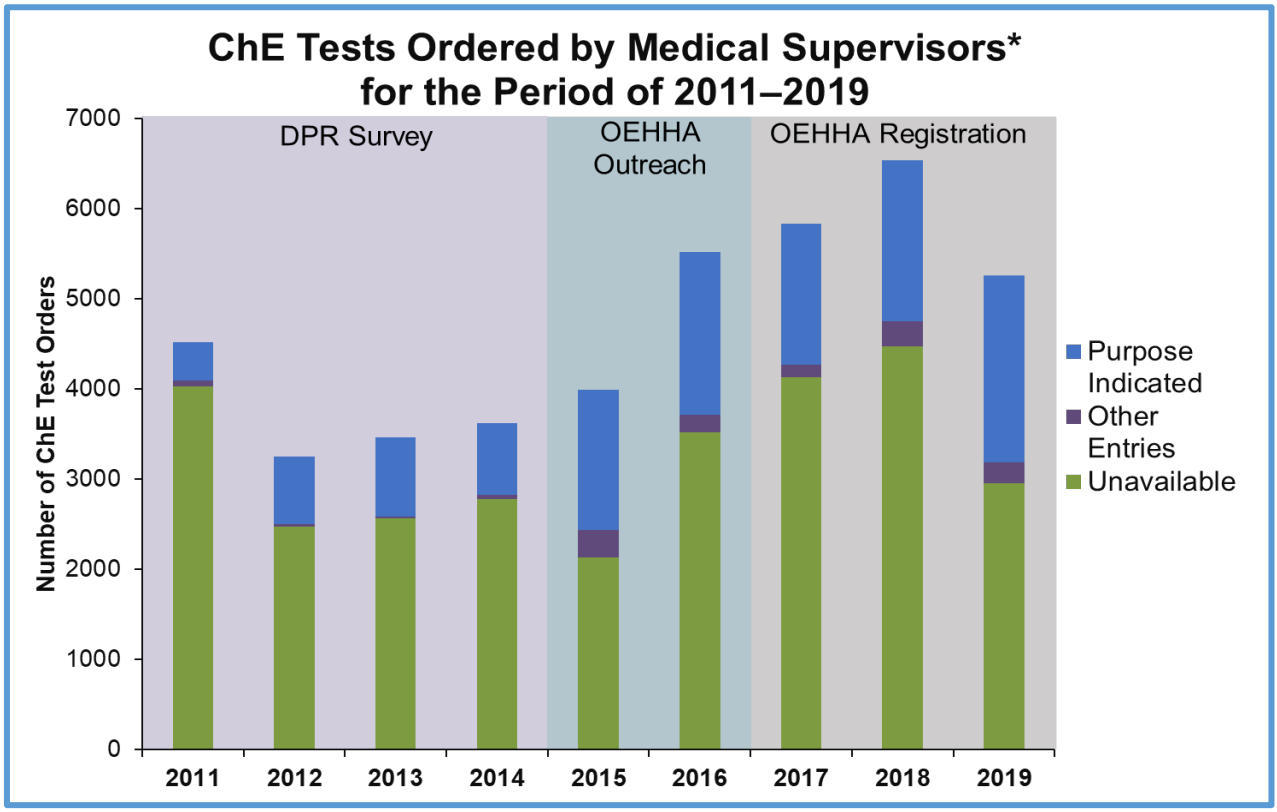


Figure 6: *Number of ChE tests ordered for known and likely agricultural employees by medical supervisors and purpose of test as indicated in the reports submitted by laboratories from 2011 to 2019. DPR identified medical supervisors in a survey from 2011 to 2014. OEHHA conducted outreach to medical supervisors from 2015 to 2016. OEHHA began registering medical supervisors in 2017. * -Physicians were identified as medical supervisors by DPR in 2014, by OEHHA's outreach project in 2015, or they were registered as medical supervisors with OEHHA (as of October 6, 2020).*

- Of the ChE tests ordered by medical supervisors, 685 (5.7%) ChE test orders from 2011–2013 and 2,594 (7.8%) from 2014–2019 were for known non-agricultural employees (e.g., HAZMAT).
- Based on the data above, there have been improvements in ChE tests ordered by medical supervisors, with the purpose of test indicated for known and likely agricultural employees, particularly in 2015 and 2016. This might be due to DPR's Medical Supervisor Survey and OEHHA's outreach efforts to identify medical supervisors.

Since DPR continues to receive ChE test results from specimens drawn throughout the state and some ChE tests ordered by medical supervisors are for non-agricultural employees (e.g., HAZMAT), the purpose of test, medical supervisor, and employer are three indicators used to identify ChE tests for the Program.

Analysis of ChE Test Results

Improvements in the Data Analysis

As mentioned in the 2015 Report, the quality of data received from reporting laboratories was poor, and thus certain steps in the data analysis had to be applied in order to allow its interpretation. Similar steps were followed in this current report including data cleaning, application of exclusion criteria and data processing to estimate baseline and calculate ChE depressions (See Appendix G). However, some changes were made to improve the overall analysis. These changes are summarized below and a comparison between the 2015 Report and the current report can be seen in Table 3.

Data Cleaning and Analysis

For the current report, OEHHA contacted physicians of individuals with significant ChE depressions to confirm the test results and identify actions taken. This process revealed some issues with the methodology used in the 2015 Report. Taking into account this new information as well as the issues already highlighted in the 2015 Report, the following four adjustments to the methodology were made (see Appendix G for details).

1. *Unique Identifiers for Individuals and Physicians*

   The R software was used to create a unique identifier for each individual and ordering physician based on name similarity and another unique identifier if available (i.e., date of birth). Based on this new approach, rows with minor typographical errors in the patient name field will still contain the same unique ID as rows with the correct patient name.

2. *Regional Analysis of Pesticide-use Data*

   Spraying seasons and correlation analysis were determined on the regional level using PUR data aggregated according to the California Agricultural Commissioners and Sealers Association Area Groups (Appendix G, Figure G3).These Area Groups were chosen because they are comprised of counties grouped into areas with similarities in agricultural practices and issues. Looking at this scale would reveal regional differences in pesticide usage.

3. *Determining Baseline Estimates from Tests taken 3 to 14 days Apart*

   For individuals with periodic testing, who had two tests taken 3 to 14 days apart during low-spraying season as recommended in the *Guidelines for Physicians*, the average of these two test results was used to estimate baseline (54%). However, in this analysis, only baseline estimates and follow-up tests within the same spraying season were used to calculate ChE depressions. This change may have excluded certain baseline estimates from the analysis but helped reduce erroneous depression calculations.

4. *Determining Baseline Estimates from Maximum Values*

   For the pool of individuals with periodic testing but without 14-day baseline estimates (46%), maximum ChE values[6] were used to extrapolate baseline estimates. In this new analysis, outliers were removed prior to this calculation, which may have led to a better estimation of ChE depressions.

Table 3: *Changes made in data cleaning and analysis since the 2015 Report.*

2015 Report	2021 Update Report
❖ Misspellings and typographical errors in individual and ordering physician names were manually corrected. ❖ For individuals with 14-day baseline estimates, baseline estimates were used if taken within the last two years. ❖ For individuals without 14-day baseline estimates, individuals' maximum ChE values were used to extrapolate baseline estimates. ❖ Statewide PUR data was used to determine low-spraying season.	❖ Unique identifiers were assigned to each individual based on similarity in names. ❖ For individuals with 14-day baseline estimates, only baseline estimates within the same year (i.e., spraying season) were used. ❖ For individuals without 14-day baseline estimates, outliers were removed prior to using maximum ChE values to extrapolate baseline estimates. ❖ Regional PUR data was used to determine low-spraying season.

Results

Patterns of ChE Activity Level

To assess if suspected handlers were being tested during spraying seasons, following HSC § 105206 requirements, OEHHA analyzed correlations between the temporal distribution of ChE test results and agricultural use of Type I and II OP/CB in high use area groups (i.e., Coast and San Joaquin Valley). As expected, suspected follow-up ChE tests from individuals with 14-day baseline estimates correlated with monthly use of Type I and II OPs/CBs (Figure 7).

[6] Maximum ChE value is an individual's highest ChE test result within a spraying season after the removal of outliers.

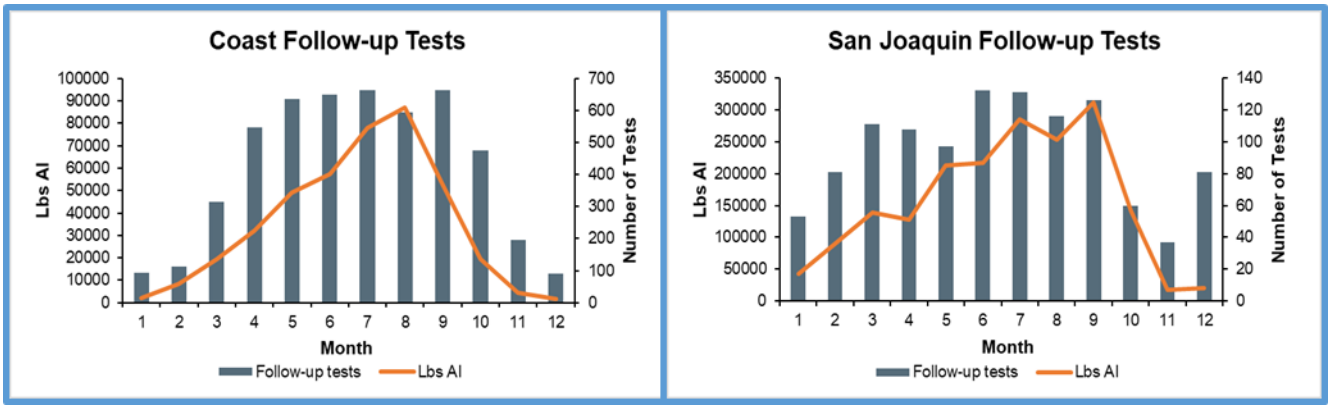


Figure 7: *Follow-up tests from Coast (left) and San Joaquin Valley (right) area group significantly correlated with monthly average PUR data (Coast: Pearson's r = 0.87, p<0.001; San Joaquin: r = 0.82, p-value = 0.001).*

Similarly, the number of ChE depressions derived from 14-day baseline estimates correlated with monthly use of Type I and II OPs/CBs for the Coast area group (Figure 8), from where the largest proportion of tests were ordered (36.7%). These observations suggest that ChE depressions occurred when pesticide usage was high, as expected, indicating that the analysis was able to retroactively determine when significant ChE depressions occurred. It should be noted that a temporal correlation was not observed between the number of ChE depressions and pesticide use in the San Joaquin Valley area group (Figure 8). A closer look at the estimated ChE depressions for each area group revealed that in April 2014, there were 12 significant ChE depressions from eight individuals in the San Joaquin Valley area group. Four of those individuals were suspected to have experienced both RBC and plasma ChE depression and were all employed by the same employer. The same analysis was conducted for individuals without 14-day baseline estimates, using individuals' maximum ChE values, and similar results were observed (Appendix G).

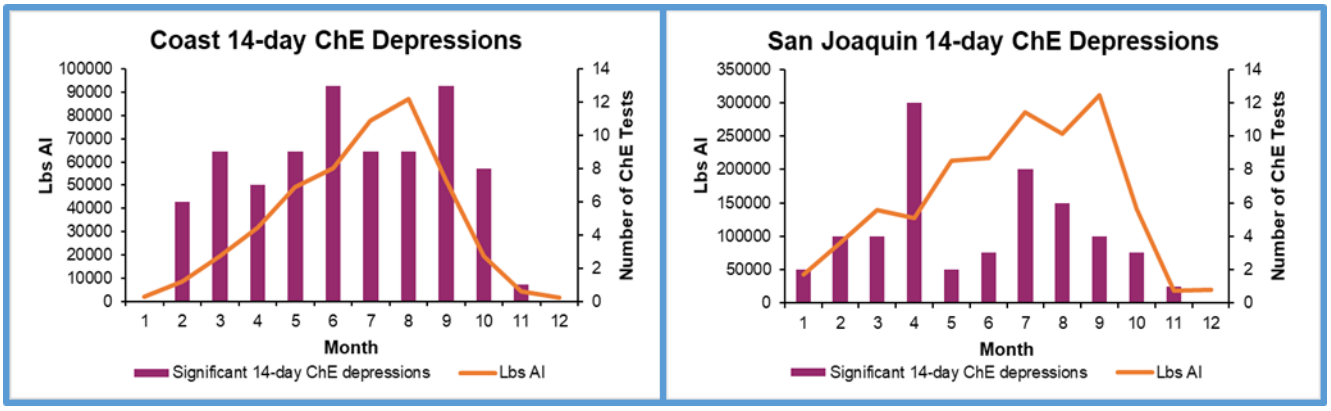


Figure 8: *ChE depressions from both Coast (left) and San Joaquin Valley area groups correlated with monthly average PUR data (Coast: Pearson's r = 0.7302, p>0.05; San Joaquin: Pearson's r = 0.45, p-value = 0.19) although it was not statistically significant for*

Participation of Workers in the Program

Similar to the 2015 Report, in an attempt to assess the degree of participation of workers in the Program, we analyzed the correlations between the spatial distribution of ChE test results and agricultural use of Type I and II OPs/CBs in the state. Both the total number of

ChE tests (Figure 9) and ChE depressions (Figure 10) significantly correlated with average pesticide usage per county.

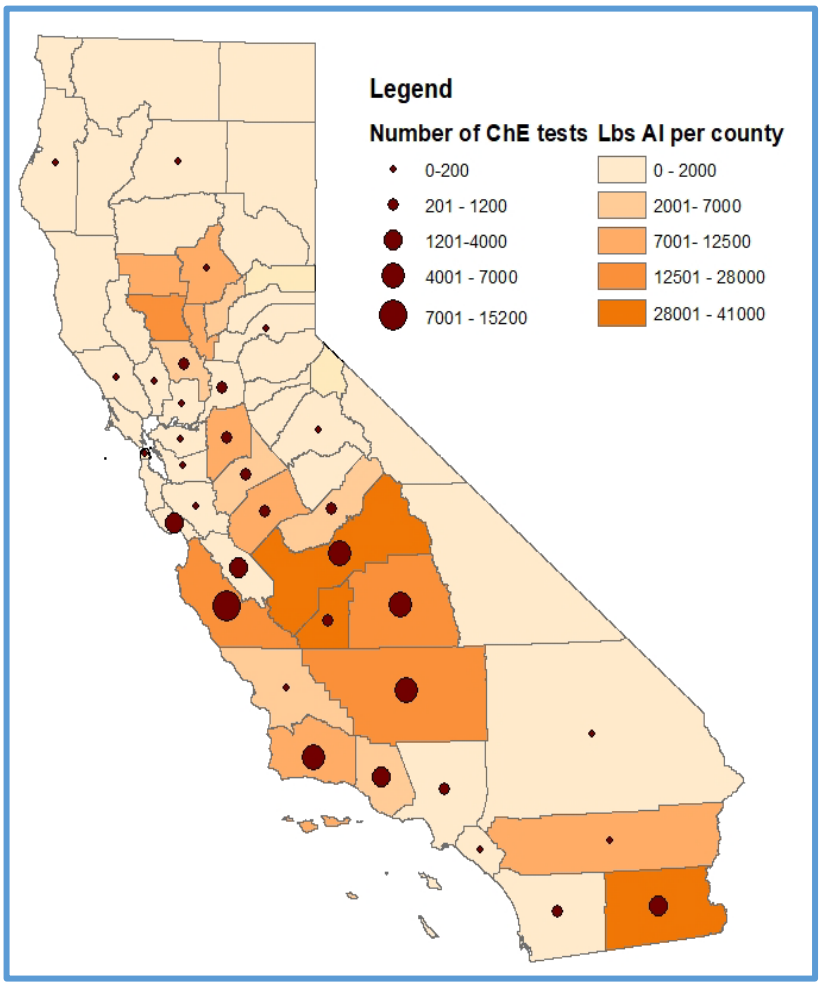


Figure 9: *Geographic distribution of Type I and II OP/CB pesticides and number of ChE tests by county across California (2014–2019). A significant correlation was determined between number of ChE tests and poundage of active ingredients used per county (Pearson's r = 0.56, p-value <0.05).*

The spatial correlation observed, along with the temporal correlation shown above, further corroborates the likelihood that ChE test results analyzed post-data cleaning and exclusion processes were indeed related to the Program and that individuals were being tested when and where pesticides were being used.

Similar to what was observed in the 2015 Report, geographic analysis revealed that in several California counties, OP/CB use did not correspond with the number of test results received. In the 2015 Report, several explanations were offered. These include:

- Several counties that had relatively high OP/CB use (e.g., northern Sacramento Valley) had very few ChE test results. A lack of test results from these counties might be due to: 1) missing location information on the ChE test reports, 2) employee's worksite and physician's location being in adjacent counties, 3) seasonal migration of workers from one county to another, 4) small farms in these areas may have hired PCBs located in other counties to

apply pesticides, 5) employers failed to follow the Program requirements, and 6) individuals in these high OP/CB use areas might not have regularly handled these pesticides. This last observation was supported by both the growers and PCB surveys (Appendix C, Figure C1).

- Other counties with no or very low pesticide usage (e.g., San Francisco) had disproportionally high number of tests. These tests were most likely from individuals not participating in the Program (e.g., pre-operative testing, Alzheimer's drug monitoring, liver disease screening, and aging research studies).

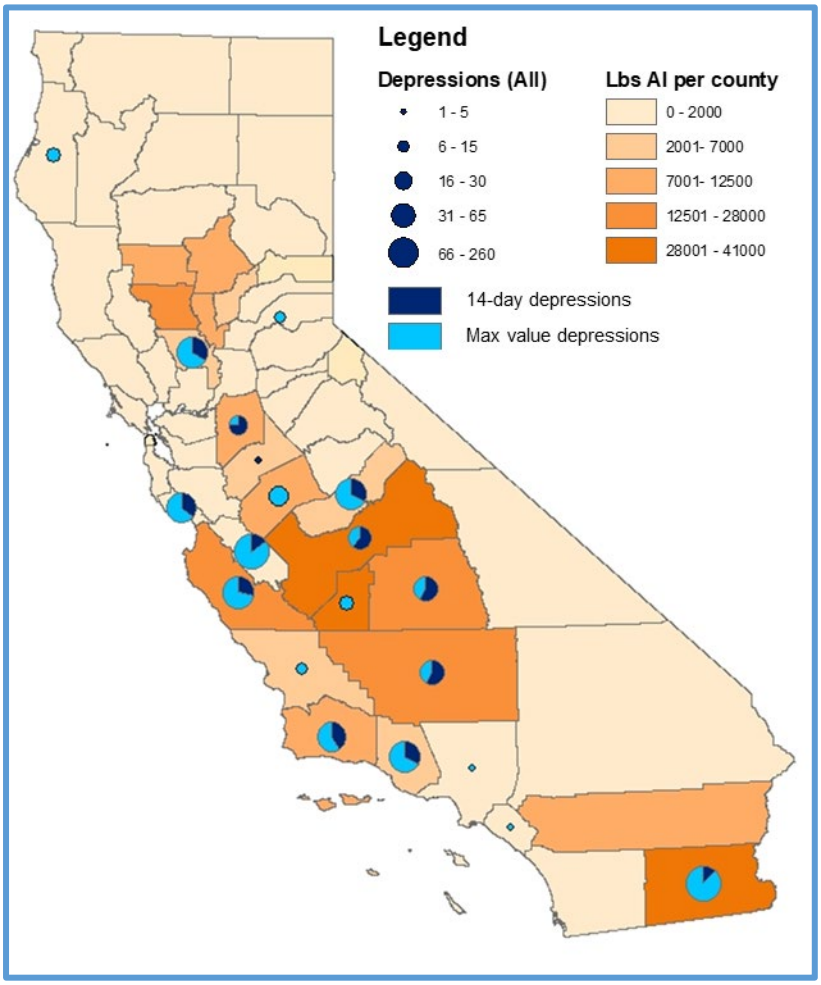


Figure 10: *Geographic distribution of Type I and II OP/CB pesticides and number of ChE depressions by county across California (2014–2019). There was a significant correlation between estimated significant ChE depressions and average poundage of active ingredients used per county (Pearson's r = 0.38, p-value <0.05). In most counties, there were more maximum ChE value depressions (light blue) than 14-day depressions (dark blue). Some ChE depressions were observed in counties that did not have high Type I and II OP/CB pesticides usage, but were adjacent to or near counties that did.*

Frequency of ChE Depressions

The number of individuals with significant ChE depressions is relatively low (2014–2019). Focusing on the individuals with 14-day baseline estimates (n =1,399), only 9.5% of individuals (n =133) experienced significant ChE depressions (<20%). Of these 9.5%, a

small proportion (18.8%, n=25) had ChE depressions that exceeded the workplace removal threshold (>30% for RBC and >40% for plasma). Furthermore, less than 1% of individuals (n=19) experienced multiple depressions and, of those, only nine experienced ChE depressions in more than one spraying season (Appendix G). Additionally, plasma ChE depressions were much more frequent than RBC ChE depressions. This was expected because plasma ChE is known to be more labile and more rapidly inactivated by pesticides so changes can be detected soon after exposure (Appendix G).

Although the trend is similar for individuals with maximum ChE value baseline estimates, the overall number of individuals with ChE depressions is higher (Appendix G). This could indicate that the maximum ChE value approach for baseline extrapolation may have led to overestimation of depressions, despite the exclusion of the outliers. This could indicate that the 14-day approach may lead to an underestimation of ChE depressions, which is an expected consequence of the new approach to estimate 14-day baseline as mentioned in the methods section above.

Data analysis showed that the proportion of individuals with significant ChE depressions (<20%) has decreased over time (Figure 11). Interestingly, a significant decline of the number of individuals with ChE depressions was observed in 2015 corresponding with the year OEHHA and DPR performed several outreach activities. ChE depressions declined between 2017 and 2019. In 2017, registration of medical supervisors was initiated, and could have contributed to the decrease in suspected ChE depressions since that year, given that medical supervisor responsibilities along with informational materials about the Program were provided during the registration process. While ChE depressions declined, the number of registered medical supervisors increased between 2017–2019. This could indicate that required actions were taken by medical supervisors and employers to protect workers from excessive exposure to Type I and II OPs/CBs. However, this could also be due to the overall decrease in pesticide usage as described in Figure 1.

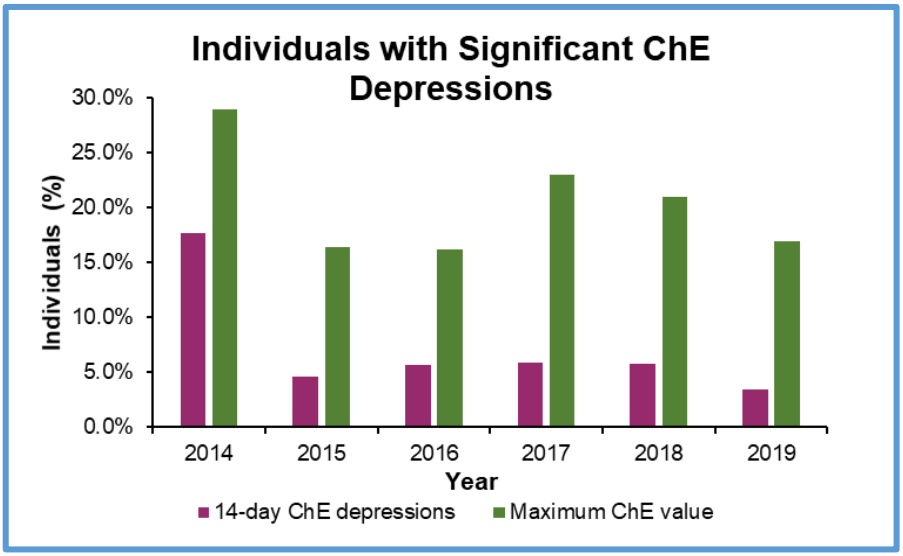


Figure 11: *Proportion of individuals with significant ChE depressions (over 20%) from 2014 through 2019.*

Conclusion of the Data Analysis

This new data analysis produced similar observations to those in the 2015 Report, and identified the following indicators of possible improvements of the Program:

- There has been a yearly increase of the proportion of ChE tests ordered by medical supervisors since 2014 (Appendix G, Figure G7).
- The number of ChE follow-up tests ordered and the number of ChE depressions correlated with Type I and II OP/CB usage per month (Figures 7 and 8).
- The number of ChE tests ordered and the number of ChE depressions correlated with Type I and II OP/CB usage per county (Figures 9 and 10).
- The proportion of individuals with significant ChE depressions has steadily decreased over the past three years (2017–2019) (Figure 11).
- Only a small proportion of individuals had multiple ChE depressions within a spraying season and an even smaller proportion had significant ChE depressions across different spraying seasons (Appendix G, Tables G3 and G4).

OEHHA and DPR have made several efforts to improve the effectiveness of the Program between 2014 and 2019. Briefly, DPR conducted surveys and completed inspections in 2014 and 2017, as well as developed outreach materials for handlers, employers, and CACs in 2018. OEHHA made outreach efforts to medical supervisors and provided information about the Program in 2015 and again in 2017, when the medical supervisor registration process was first initiated. These efforts may have helped to improve the understanding of the Program by the medical supervisors and employers, and led to better compliance, which may have been reflected in the findings discussed above.

Level of Awareness of the Program by Employers (Agricultural PCBs and Growers)

In 2017, DPR conducted an inspection and survey of agricultural PCBs to determine the industry's knowledge of and compliance with the specific requirements of 3 CCR § 6728. A similar project in 2014 targeted agricultural growers. Both agricultural growers and PCBs apply pesticides to treat crops and commodities. However, PCBs' primary operation is to apply pesticides to control and mitigate pests, whereas the growers' scope of operations is exclusively agricultural, and includes other field activities such as planting and harvesting.

Although growers apply pesticides only within their respective agricultural fields, and the PCBs conduct pesticide applications beyond farms, both the growers and PCBs implement similar business practices to comply with the Program requirements. As an example, some growers and PCBs in the survey indicated they rotated task assignments of their handlers such that these workers did not handle

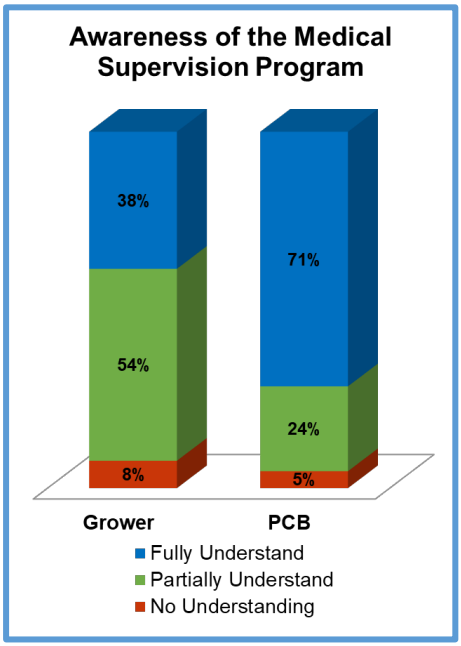


Figure 12: *Growers (n=26) and PCB's (n=21) level of understanding of the Program.*

ChE-inhibiting pesticides more than six days in a given 30-day period. By doing so, the employers were not required to have a medical supervision program.

- Of the 42 PCBs surveyed and inspected, 50% (21) had employees who were under the Program.
- Whereas only a third (26) of the 83 growers surveyed and inspected had employees that met the Program criteria, indicating PCBs may have more employees that regularly handle OPs and CBs due to the nature of their business.
- Consistent with the ChE test results and PUR data, the distribution of the growers and PCBs under the Program were concentrated in the central and southern regions of California.

Even though both the growers and the PCBs were aware of the Program, the PCBs had a better understanding of its specific requirements (Figure 12). For example, most PCBs could explain "regularly handling" in their own words. Most of the PCBs (86%) retained OP/CB use records as well as their employee's records related to the Program[7] as compared to only 62% the growers (Figure 13).

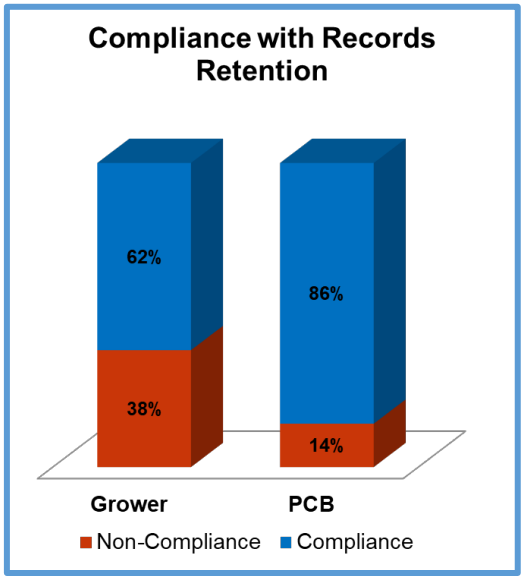


Figure 13: *Percentage of growers (n=26) and PCBs (n=21) who retained records as required by the Program.*

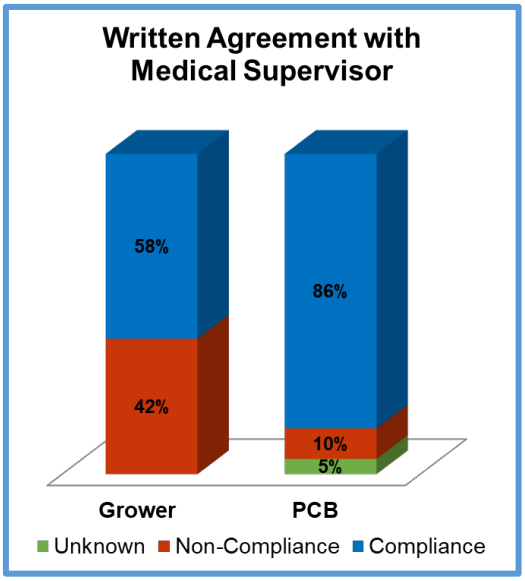


Figure 14: *Percentage of growers (n=26) and PCBs (n=21) who have a written agreement with a medical supervisor.*

Similarly, the majority of the PCBs surveyed had an agreement with a medical supervisor to provide monitoring of the ChE levels of their regular handlers, but only slightly more than half of the growers had such an agreement (Figure 14). Of the 21 PCBs under the Program, 17 (81%) sent their employees for ChE level monitoring as determined by the medical supervisor or by themselves (PCB employer or owner) if no recommendations were made by the medical supervisor. However, with the growers, it remained uncertain whether they

[7] 3 CCR § 6728(c)(3) states employer shall keep a record of the agreement with medical supervisor, OP/CB use records, all recommendations received from the medical supervisor, and all employees ChE test results for 3 years.

themselves (growers) or their respective medical supervisor decided on the frequency of ChE testing for employees under the Program.

Three PCBs each reported having one employee whose ChE test results fell below 80%, as compared to their baseline levels. The PCBs acknowledged investigating employees' work practices due to the employees' ChE test results meeting this threshold. Two handlers, working for different PCBs, were removed from the exposure source when their RBC ChE activity level fell to 70% or lower, or their plasma ChE activity level fell to 60% or lower. Only one grower had an employee whose ChE test results were below the threshold. However, the medical supervisor informed the grower that his employee's ChE test results were physiologically low and were not due to an exposure. This grower not only investigated the employee's work practices but also modified the employee's work duties.

The findings from these surveys suggest continued outreach to both growers and PCBs is needed to strengthen their knowledge and understanding of the Program, and their compliance with its requirements. In addition to the distribution of outreach materials to workers and workers at agricultural events, these materials could also be distributed by the CAC when these businesses apply for OPs/CBs use permit.

SUMMARY OF FINDINGS AND FUTURE DIRECTIONS

In this report, DPR and OEHHA evaluated the effectiveness of the Program and the utility of the laboratory-based reporting of ChE for pesticide-related illness surveillance and prevention using data obtained from:

- Information derived from the ChE test results.
- Registration of medical supervisors.
- Information obtained from a pest control business survey and inspections.

Effectiveness of the Program

Overall, similar to conclusions in the 2015 Report, the Program appeared effective between 2014 and 2019 in protecting agricultural workers handling ChE-inhibiting pesticides. Although there were some improvements in the data quality observed since 2014, certain assumptions and criteria still had to be applied to identify ChE test results for individuals likely under the Program. Although complete information has been difficult to obtain, DPR and OEHHA were able to identify individuals as part of the Program and estimate CHE depressions. The analysis of the ChE data indicates that most individuals identified as part of the Program did not have significantly depressed ChE activity levels. Moreover, most of the physicians who regularly ordered ChE tests were medical supervisors, a marked improvement from 2014. However, due to the frequency of the submission of the ChE test reports by the laboratories and level of processing required of the data, real-time analysis and detection of individuals with depressed ChE activity levels are not feasible.

The findings from the agricultural PCB survey and inspections concurred with the findings from the Growers' Focused Headquarters' Inspection, whereby the PCBs surveyed were familiar with the Program but had varying levels of understanding of the specific requirements. Both growers and PCBs reported conducting workplace practice investigations, including removal from handling OPs/CBs as outlined in the Program. However, these actions were not able to be confirmed due to the delay of information.

As mentioned in the previous report, since the medical supervisors are responsible for several facets of the Program (e.g., evaluating the employee, submitting ChE test laboratory requisition forms, receiving and evaluating ChE test results from the laboratory, and informing the employee and the employer of the test results), it may make sense to also transfer the ChE reporting responsibility to the medical supervisor. This requirement would allow targeted education efforts to one group, and could facilitate more complete and timely reporting, enabling prompt data analysis, evaluation, and the determination of action levels when necessary.

In general, outreach efforts by DPR and OEHHA to Program participants have resulted in improvements in the quality of the ChE reports received, and their understanding of the Program. Further enhancement of educational materials and outreach efforts to improve communications among all Program participants would strengthen efforts to monitor the Program's effectiveness to enhance protection of California's agricultural workers.

Utility of Laboratory-Based Electronic Reporting

Cholinesterase test results reporting is a complex mechanism that necessitates a thorough understanding of the Program's requirements by all individuals involved in each step of the ChE reporting process. The data provided by the ordering medical supervisor, the transfer of data from the blood-drawing facility to the laboratory performing ChE analysis, and the reporting by the laboratory to DPR all have to work in union in order to provide the data required under HSC § 105206.

Electronic reporting of ChE data from laboratories provided OEHHA with names of physicians to register as medical supervisors, and allowed for the identification of data patterns and gaps. ChE reporting significantly improved after 2015 potentially due to DPR's Medical Supervisor Survey in 2014, OEHHA's outreach in 2015, and OEHHA's registration process since 2017. There have also been improvements in data quality and quantity in the last few years. As compared to ChE test results received from reporting laboratories from 2011–2013, there was a two-fold increase in the number of ChE test results with the purpose of test indicated in the 2014–2019 dataset. There was also a two-fold increase in the number of ChE test orders from medical supervisors for known and likely agricultural employees from 2014–2019 as compared to 2011–2013. Of the ChE tests ordered from medical supervisors for known or likely agricultural employees from 2014–2019, there was a four-fold increase in the identification of the purpose of the test. However, the data quality could be further improved as 59.8% of ChE test orders submitted by reporting laboratories to DPR from 2011–2019 do not appear to be related to the Program and/or lacked the required data elements under HSC § 105206. It is not certain whether all ChE tests ordered by medical supervisors from 2011–2019 were for employees under the Program because 72.7% of ChE test orders did not indicate the purpose of test using terms related to the Program. The absence of these required data elements may indicate either: 1) ChE test results were for individuals under the Program but had missing data, or 2) ChE test results were for individuals not under the Program.

Further Improvements

Gaps remain in the information that laboratories receive from ordering medical personnel, and errors can be introduced from the laboratories. DPR is proactively working to address:

- Missing information on the test purpose and other required data elements that limit the utility of ChE test results for evaluating the effectiveness of the Program.
- Improvements in data quality (e.g., data entry errors, discrepancies).
- Medical supervisor and employer information to definitively determine if the ChE tests were related to the Program.

Related to this, ChE test reports from laboratories have been useful in identifying which ordering physicians are medical supervisors in order to inform them about the Program's registration process and conduct targeted outreach and training. Every year, prior to registration renewal deadline, OEHHA follows up with physicians to determine whether additional medical supervisors can be identified. Additionally, OEHHA will investigate the reasons for lack of reporting to local health officers to ensure compliance with reporting under AB 2892.

The reporting laboratories submit their ChE test results in batches, at times several months after the blood specimen has been analyzed. Therefore, due to the frequency of the submission of the ChE test reports by the laboratory and level of processing required of the data, analysis and real-time detection of individuals with depressed ChE activity levels are not feasible. DPR continues to be proactive in monitoring data submitted by reporting laboratories and working with their personnel to obtain accurate data and information as required by law. Consequently, reporting laboratories have been responsive to DPR inquiries and have corrected information when possible. Likewise, identifying the missing information can help OEHHA focus its effort in training registered medical supervisors. To ensure data consistency in reporting and improve data quality, DPR and OEHHA will explore the possibility of utilizing a sole, state-run (CDPH) laboratory to analyze ChE tests ordered by medical supervisors and the possibility for employers or medical supervisors to submit test results and recommendations directly to DPR/OEHHA.

Future Directions

Electronic-based reporting gives DPR and OEHHA the ability to analyze test results on a statewide scale. Surveys and outreach efforts provided additional insight into the Program. The information from these various components helped identify program strengths as well as elements in need of further improvement. Although the recommendations proposed in the 2015 Report were carried out and provided some useful information, similar shortcomings were identified in this update report. Along with current ongoing activities, DPR and OEHHA plan to take the following steps to help enhance the Program's effectiveness and utility of laboratory-based reporting (Table 4):

***Table 4**: DPR and OEHHA future directions.*

DPR/OEHHA – Future Directions	Leads/ *Participants*	Requires Legislation?
• Focus the next evaluation on counties with high OP/CB use and conduct a monitoring study. *Rationale: to evaluate the components of the Program on a smaller scale to better assess its effectiveness.*	Leads: DPR, OEHHA	No
• Amend CCR Title 3 section 6728 (c)(1) to add the recommended time frame for performing ChE baseline testing for workers under the Program. *Rationale: to align the requirements for employers with OEHHA's Guidelines for Physicians.*	Lead: DPR	No
• Amend HSC § 105206 to request additional data elements from reporting laboratories to better identify workers and ordering physicians. *Rationale: to help better identify individuals under the Program.*	Leads: DPR, OEHHA	Yes

On-going Activities by DPR and OEHHA

- Continue registering physicians and updating outreach materials for physicians.
  - Follow-up with medical supervisors who did not indicate the purpose on ChE test results.
  - Prepare and distribute factsheet for physicians on the requirements of HSC § 105206.
- Distribute outreach materials to employers and CACs.

GLOSSARY OF TERMS

3 CCR § 6728: Title 3, section 6728 of the California Code of Regulations, on Medical Supervision

AB 1963: Assembly Bill that added the Health and Safety Code section 105206 requiring California Department of Public Health-approved laboratories to submit cholinesterase test results of workers under the medical supervision program to the Department of Pesticide Regulation. AB 1963 was signed by the governor in September 2010 and became law on January 1, 2011.

Accession Number: A unique number assigned by the laboratory to each blood specimen submitted for analysis. The accession number protects a patient's privacy by functioning as a unique identifier rather than using the patient's name or other personal identifier.

Action Levels: A depression in the level of cholinesterase activity that meets one of the following thresholds:

- If either red blood cell or plasma cholinesterase is depressed below 80% of the baseline (that is, more than 20% depression from the baseline), it triggers a reassessment of work activities.
- If a worker's cholinesterase level drops more than 30% from the red blood cell baseline or more than 40% from the plasma baseline, he/she is removed from the exposure source.
- Following a worker's removal, his/her red blood cell and plasma cholinesterase must be monitored, and he/she is not allowed to work with or handle Toxicity Categories I and II organophosphate and carbamate pesticides until red blood cell and plasma cholinesterase levels return to at least 80% of the baseline.

Baseline: Red blood cell and plasma cholinesterase determinations measured prior to an employee's exposure to Toxicity Categories I and II organophosphate and carbamate pesticides. By regulation, a baseline cholinesterase test is required of all employees who will "regularly handle" these pesticides regardless of the frequency of subsequent monitoring. Once the baseline is determined, subsequent test results are evaluated as a percentage of the baseline activity.

Carbamate (CB): An organic compound with structural features that result in inhibition of cholinesterase enzymes, which are critical to normal function of the nervous system. Aldicarb, carbofuran, carbaryl and methomyl are examples of carbamate pesticides.

CDPH: California Department of Public Health

Cholinesterase (ChE): An enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine, and helps the nervous system to work properly. Under the Medical Supervision Program, two types of cholinesterase (plasma and red blood cell (RBC)) are required to be measured for all covered employees to account for the differences in the mode of action of cholinesterase-inhibiting pesticides.

- **Plasma Cholinesterase**: Considered to be more labile than red blood cell cholinesterase and thus less reliable in reflecting actual enzyme depression

at neuro-effector sites. It is generally more rapidly inactivated by exposure to organophosphates/carbamates.

- **RBC Cholinesterase:** Biochemically the same enzyme as the acetylcholinesterase located at the neuro-effector cell synapses. It is often depressed more slowly than plasma cholinesterase by exposure to organophosphates/carbamates.

County Agricultural Commissioner (CAC): Primary enforcement agents, at county level, for the State pesticide laws and regulations.

DPR: Department of Pesticide Regulation, a department of the California Environmental Protection Agency.

Drawing Facility or Laboratory: Any laboratory that collects specimens (i.e., draws blood) from tested persons. Although these laboratories perform basic analyses, they send complex or infrequently ordered laboratory tests to a reference laboratory for analyses.

Guidelines for Physicians: The document, *Guidelines for Physicians Who Supervise Workers Exposed to Cholinesterase-Inhibiting Pesticides*, prepared by the Office of Environmental Health Hazard Assessment. This handbook describes the Medical Supervision Program and the responsibilities of the medical supervisors. The 6th edition of this document was released in 2017.

Handler: Any person who:

i. Mixes, loads, transfers, or applies pesticides.
ii. Cleans, adjusts, handles, or repairs the parts of mixing, loading, or application equipment that may contain pesticide residue.
iii. Acts as a flagger.

HSC § 105206: Health and Safety Code section 105206, codified into law by the enactment of AB 1963, that took effect on January 1, 2011. AB 2892 (Statutes of 2016, Chapter 475) took effect in January 2017. AB 3220 (Statutes of 2020, Chapter 296) goes into effect January 1, 2021 and extended the sunset until January 1, 2023, and as of that date is repealed, unless a later statute enacted before January 1, 2023, deletes or extends that date.

Laboratory Requisition Slip: Form provided by the laboratories for ordering physicians to use when submitting specimen samples for analysis.

Medical Supervisor: Under HSC § 105206, a licensed physician (M.D. or D.O.) who has a written agreement with employers of agricultural workers who regularly apply cholinesterase- inhibiting pesticides in Toxicity Categories I and II, to examine the employees for fitness, order cholinesterase tests, and to make the necessary recommendations based on the results of an employee's cholinesterase test results.

OEHHA: Office of Environmental Health Hazard Assessment, a department of the California Environmental Protection Agency.

Organophosphate (OP): A general term for esters of phosphoric acid that constitute the common structural element of many insecticides. These pesticides are toxic because they inhibit cholinesterase enzymes and impair normal function of the nervous system. Organophosphates are a large class of pesticide products; examples include parathion, malathion, chlorpyrifos, and naled.

Pesticide Use Report (PUR): A comprehensive report of all agricultural pesticide use in California. Use data must be submitted monthly to County Agricultural Commissioners, who in turn, report the data to the Department of Pesticide Regulation.

"Program": Medical Supervision Program (3 CCR § 6728) as used in this document.

Purpose of Test: Under HSC § 105206, a medical supervisor must indicate on the test order the reason for ordering cholinesterase tests for an employee.

- **Baseline**: pre-exposure test ordered to establish the normal ChE activity level of a worker under medical supervision.
- **Follow-up**: test ordered for periodic testing/follow-up assays of a worker under medical supervision.
- **Suspected illness**: Test ordered when there are identified effects of a suspected or reported pesticide exposure.
- **Recovery***:* Retest ordered after a suspected illness or exposure. This test is recommended to be done weekly, until both plasma and RBC ChE activity levels returned to 80% or more of the worker's own baseline ChE test.

"Regularly handle": Employees who handle pesticides any part of the day for more than six calendar days in any 30-day qualifying period beginning on the first day of handling (3 CCR § 6000).

Reporting Laboratory: Also called reference laboratory, this is an independent referral or diagnostic facility equipped with state-of- the-art equipment, and trained personnel to conduct various types of tests not otherwise available in most laboratories. Hospitals, laboratories, and physicians will often use a reference laboratory for more complex or less frequently utilized tests.

Signal Word: One word used to indicate the acute toxicity of the formulated pesticide product.

i. **Danger**: Highly toxic by at least one route of exposure.
ii. **Warning**: Moderately toxic if ingested, absorbed through the skin, or inhaled.
iii. **Caution**: Slightly toxic if eaten, absorbed through the skin, or inhaled.

Toxicity Categories I and II (Type I and II): Refers to U.S. Environmental Protection Agency's classification system for pesticides that addresses the acute toxicity of these products.

i. **Toxicity Category I**: Highly toxic; Signal word "Danger."
ii. **Toxicity Category II**: Moderately toxic; Signal word "Warning."

Appendix A: Background

1. California Code of Regulations, Title 3, Section 6728. Medical Supervision

(a) Whenever an employee mixes, loads, or applies a pesticide with the signal word "DANGER" or "WARNING" that contains an organophosphate or carbamate, for the commercial or research production of an agricultural plant commodity, the employer shall maintain use records that identify the employee, the name of the pesticide, and the date of use. The original or copies of documents otherwise required to be maintained by this chapter may be used to meet the requirements of this Section provided they contain the information required by this Section.

(b) Each employer who has an employee who regularly handles pesticides specified in (a) shall have a written agreement signed by a physician, that includes the names and addresses of both the physician providing the medical supervision and the employer responsible for the employees, stating that the physician has agreed to provide medical supervision and that the physician possesses a copy of, and is aware of the contents of the document "*Medical Supervision of Pesticide Workers-Guidelines for Physicians*" (available from the Office of Environmental Health Hazard Assessment). A copy of this agreement shall be given to the commissioner by the employer no later than when an employee begins to regularly handle pesticides specified in (a).

(c) The employer's responsibilities for medical supervision for employees regularly handling pesticides specified in (a) shall include the following:

(1) All covered employees shall have baseline red cell and plasma cholinesterase determinations. Baseline values shall be verified every two years. For new employees, the medical supervisor may accept previously established baseline values if they are obtained in accordance with these regulations by the same laboratory methodology and are acceptable to the laboratory which will analyze the new employee's blood samples.

(2)(A) The employer shall ensure that each employee, not previously under medical supervision associated with that employer, has red cell and plasma cholinesterase determinations within three working days after the conclusion of each 30-day period in which pesticides specified in (a) are regularly handled.

(B) After three tests at 30-day intervals, further periodic monitoring shall be at intervals specified in writing by the medical supervisor except for verification of baseline as specified in (1).

(C) Where the medical supervisor has made no written recommendation for continued periodic monitoring, the testing interval shall be 60 days.

(3) The employer shall keep a record of the agreement to provide medical supervision, use records, all recommendations received from the medical supervisor, and all results of cholinesterase tests required to be made on his/her employees by this Section or by the medical supervisor. Records required by this Section shall be maintained for three years and shall be available for inspection by the employee, the Director, commissioner, county health official, or state health official.

(4) The employer shall follow the recommendations of the medical supervisor concerning matters of occupational health.

(5) The employer shall post the name, address, and telephone number of the medical supervisor in a prominent place at the locale where the employee usually starts the workday; or if there is no locale where the employee usually starts the workday, at each worksite; or in each work vehicle.

(d) The employer shall investigate the work practices of any employee whose red cell or plasma cholinesterase levels fall below 80 percent of the baseline. The investigation of work practices shall include a review of the safety equipment used and its condition; and the employee's work practices which included employee sanitation, pesticide handling procedures, and equipment usage. The employer shall maintain a written record of the findings, any changes in equipment or procedures, and any recommendations made to the employee.

(e) The employer shall remove an employee from exposure to organophosphate or carbamate pesticides if the employee's plasma cholinesterase level falls to 60 percent or less of baseline, or if red cell cholinesterase falls to 70 percent or less of baseline. The employee shall be removed from further exposure until cholinesterase values return to 80 percent or more of their respective baseline values. The employer shall maintain written records of the dates of removal and the dates when employees are returned to exposure.

(f) To meet the requirements of these regulations, acetylcholinesterase (also known as red blood cell cholinesterase) and butyrylcholinesterase (also known as plasma or serum cholinesterase or pseudocholinesterase) tests ordered by a medical supervisor for occupational health surveillance shall be performed by a clinical laboratory currently approved by the State Department of Health Services to perform these tests. By January 1, 2000, tests shall be performed according to the procedures outlined below. If tests cannot be performed according to the following procedures, the conversion procedure outlined in 3 CCR §6728 (f)(8) shall be performed.

(1) Using personnel and procedures acceptable to the Department of Health Services (Business and Professions Code sections 1242,1243,1246,1269,2070; Health and Safety Code sections 120580, 1607), blood collection and storage shall be done according to the following conditions:

(A) Blood samples shall be kept in ice or at a temperature of 4º C until time of assay. If the sample is centrifuged to remove the erythrocytes from the plasma, the plasma shall be stored frozen at a temperature of minus 20º C until the assay is performed. If possible, the assay shall be performed within 24 hours after blood collection. Time of sample collection, analysis, and storage conditions shall be specified on the report.
(B) Ethylenediaminetetraacetic acid (EDTA) or heparin shall be used as an anticoagulant in a standard vacutainer tube.

(2) The reagents and equipment shall conform to the following conditions:

(A) A spectrophotometer at a wavelength between 405 and 425 nanometers shall be used.
(B) The assay shall be performed at a temperature of 25º C.

(C) The following conditions regarding the buffer/chromogen shall apply:

1. A sodium phosphate buffer shall be used at a concentration of 0.1 M adjusted to a pH of 8.0 with a pH meter calibrated at both 7.0 and 10.0.
2. Dithiobisnitrobenzoic acid (DTNB) at a stock concentration of 9.7 mM in 0.1 M sodium phosphate buffer pH 7.0 shall be used.

(D) The substrate acetylthiocholine iodide shall be used at a stock concentration of 10.1 mM in 0.1 M sodium phosphate buffer pH 8.0.

(E) The butyrylcholinesterase inhibitor quinidine hydrochloride monohydrate shall be used at a stock concentration of 6 mM in distilled deionized water.

(3) The acetylcholinesterase enzyme assay shall be performed within 15 minutes of preparation and the procedure for performing the assay shall be as follows:

(A) Measure 0.2 mL whole blood and add into a 1.8 mL solution of deionized distilled water; mix thoroughly and keep the solution on ice.

(B) To 2.5 mL of the sodium phosphate buffer, add 0.02 mL of the blood solution, 0.1 mL of DTNB (0.32 mM final concentration) and 0.1 mL of quinidine (0.2 mM final concentration); mix thoroughly and allow to sit for 5 minutes.

(C) Add 0.3 mL acetylthiocholine iodide (1.0 mM final concentration) into the buffer/sample solution and mix thoroughly.

(D) Measure absorbance over the linear portion of the enzyme activity curve in the spectrophotometer.

(4) The procedure for performing butyrylcholinesterase enzyme assay determination shall be as follows:

(A) Physical separation of plasma or serum shall be performed.

(B) If samples are frozen, they shall be thawed at room temperature to assure homogeneity of the sample.

(C) To 2.6 mL of the sodium phosphate buffer, add 0.02 mL of the plasma or serum and 0.1 mL of DTNB (0.32 mM final concentration), mix thoroughly and allow to sit for 5 minutes.

(D) Add 0.3 mL acetylthiocholine iodide (1.0 mM final concentration) into the buffer/sample solution and mix thoroughly.

(E) Measure absorbance over the linear portion of the enzyme activity curve in the spectrophotometer.

(5) A Buffer Blank containing 2.6 mL of sodium phosphate buffer, 0.3 mL of acetylthiocholine (1.0 mM final concentration), and 0.1 mL of DTNB (0.32 mM final concentration) and 0.02 mL of distilled deionized water shall be run with every batch of assays.

(6) Reporting units shall be in International Units per milliliter of sample (IU/mL).

(7) Baseline and follow up assays specified in 3 CCR §6728 (c)(2)(A) shall be conducted by the same laboratory method. If an assay different from that described above is used, the method shall be shown comparable with the foregoing conditions and a conversion equation prepared. Results shall be reported in International Units

per mL on both the original and the converted scale. The conditions to establish comparability shall be as described below.

(A) Using personnel and procedures acceptable to the Department of Health Services (Business and Professions Code sections 1242, 1243, 1246, 1269, 2070; Health and Safety Code sections 120580, 1607), blood samples shall be collected from at least ten subjects.

(B) Blood from each subject shall be tested by serial dilution as specified in "Comparison of Acetylcholinesterase Assays Run under Conditions Specified by the Standard Ellman Method and Conditions Specified by a Commercial Cholinesterase Reagent Kit." HS-1752, July 30, 1998, Department of Pesticide Regulation, Worker Health and Safety Branch.

(C) Test dilutions shall be made at 100% and 50% of enzyme activity.

(D) Triplicate samples shall be run by both the reference and the alternative methods.

E) Pearson product-moment correlation coefficient squared (r^2) shall be at least 0.9 between results of the alternative and reference methods.

Note: Authority cited: section 12981, Food and Agricultural Code.
Reference: Sections 12980 and 12981, Food and Agricultural Code; and Section 105206, Health and Safety Code.

2. California Health and Safety Code Section 105206

(a) In order for an employer to satisfy his or her responsibilities for medical supervision of his or her employees who regularly handle pesticides pursuant to Section 6728 of Title 3 of the California Code of Regulations, the employer shall contract with a medical supervisor registered with the Office of Environmental Health Hazard Assessment (OEHHA).

(b) A laboratory that performs tests ordered by a medical supervisor shall report the information specified in subdivision (c) to the Department of Pesticide Regulation. Reports shall be submitted to the Department of Pesticide Regulation on, at a minimum, a monthly basis. For the purpose of meeting the requirements in subdivision (e), the reports shall be submitted via electronic media and formatted in a manner approved by the director. The Department of Pesticide Regulation shall share information from cholinesterase reports with the Office of Environmental Health Hazard Assessment (OEHHA) and the State Department of Public Health on an ongoing basis, in an electronic format, for the purpose of meeting the requirements of subdivisions (f) and (g).

(c) The laboratory shall report all of the following information in its possession in complying with subdivision (a):

(1) The test results in International Units per milliliter of sample (IU/mL).

(2) The purpose of the test, as indicated by the medical supervisor, as a cholinesterase test requested for an agricultural worker under medical supervision, and, if so, whether it is for a baseline, followup, or recovery test ordered to meet the requirements of Section 6728 of Title 3 of the California Code of Regulations or for the evaluation of suspected pesticide illness.

(3) The name of the person tested.

(4) The date of birth of the person tested.

(5) The name, address, and telephone number of the medical supervisor who ordered the analysis.

(6) The name, address, and telephone number of the laboratory.

(7) The date that the sample was collected from the person and the date the result was reported.

(8) Contact information for the person tested and his or her employer, if known and readily available.

(d) The registered medical supervisor ordering a cholinesterase test for a person pursuant to subdivision (a) shall note in the test order the name of the medical supervisor and the purpose of the test, pursuant to paragraph (2) of subdivision (c), and ensure that the person tested and the employer receive a copy of the cholinesterase test results and any recommendations from the medical supervisor based upon those results within 14 days of the medical supervisor's receipt of the results. The medical supervisor shall report any worker with cholinesterase depression indicating pesticide exposure to the local health officer pursuant to Section 105200.

(e) All information reported pursuant to this section shall be confidential, as provided in Section 100330, except that the OEHHA, the Department of Pesticide Regulation, and the State Department of Public Health may share the information for the purpose of

surveillance, case management, investigation, environmental remediation, or abatement with the appropriate county agricultural commissioner and local health officer.

(f) The OEHHA shall establish a procedure for registering and deregistering medical supervisors for the purposes of outreach and training and may establish reasonable requirements for performance. The OEHHA shall review the cholinesterase test results and may provide an appropriate medical or toxicological consultation to the medical supervisor. In addition to the duties performed pursuant to Section 105210, the OEHHA, in consultation with the Department of Pesticide Regulation and the local health officer, may provide medical and toxicological consultation, as appropriate, to the county agricultural commissioner to address medical issues related to the investigation of cholinesterase inhibitor-related illness.

(g) The Department of Pesticide Regulation and the OEHHA shall prepare and publicly post an update on the effectiveness of the medical supervision program and the utility of laboratory-based reporting of cholinesterase testing for illness surveillance and prevention by January 1, 2021.

(h) This section shall remain in effect only until January 1, 2021, and as of that date is repealed, unless a later enacted statute, that is enacted before January 1, 2021, deletes or extends that date.

3. Recommendations and Future Directions from the 2015 Report and Current Status

DPR/OEHHA Recommendations	Leads/ *Participants*	Requires Legislation?	Status
• Transferring cholinesterase reporting responsibilities from the laboratories to the medical supervisors may ultimately be a more efficient way to implement the Program.	Leads: DPR, OEHHA	Yes	On-going
• The cholinesterase reporting should continue at least through December 31, 2018 in order to obtain additional data with clearer information on the purpose of the test and to allow further evaluation of the Program.	Leads: DPR, OEHHA *Participant: CDPH*	Yes	Completed

DPR/OEHHA Future Directions	Leads/ *Participants*	Requires Legislation?	Status
• Enhance outreach and training to increase understanding of the Program by the medical supervisors, employers, laboratories, and the County Agricultural Commissioner staff.			
❖ Develop materials and conduct outreach efforts for the employers on their roles and responsibilities under the Program, such as, record retention of employees' cholinesterase test results and medical supervisor recommendations.	Lead: DPR *Participant*: *CAC*	No	Completed
❖ Promote and expand the medical supervision training, emphasizing the provisions of HSC §105206 and continuing in-person visits to the medical supervisors.	Lead: OEHHA	No	Completed
❖ Conduct focused headquarters inspections of Pest Control Operators similar to those that DPR conducted with growers.	Lead: DPR *Participant*: *CAC*	No	Completed
❖ Increase the County Agricultural Commissioners' awareness of the Program; include a module on the Program during Enforcement Training.	Lead: DPR *Participant*: *CAC*	No	On-going
❖ Coordinate with CDPH on outreach efforts to the laboratories. Develop clear requisition slips that require indication of the purpose of the cholinesterase test.	Lead: CDPH *Participant*: *DPR*	No	Completed
• Continue coordination between DPR, OEHHA and CDPH to enhance the effectiveness of the Program			
❖ Continue coordination between DPR, OEHHA and CDPH to enhance the effectiveness of the Program	Lead: DPR *Participants*: *CDPH, OEHHA*	No	Completed
❖ Develop a list of currently active medical supervisors and update it regularly.	Lead: OEHHA	No	Completed

Appendix B: DPR Outreach Materials

1. Extra Medical Care for Handlers Who Use Organophosphate and Carbamates

PESTICIDE SAFETY Information

dpr

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

A No. 10

Extra Medical Care For Handlers Who Use Organophosphates and Carbamates

If you mix, load, or apply organophosphate or carbamate pesticides, you might need extra medical care. Your employer must track how often you work with these pesticides. Your employer must arrange for you to get extra medical care if:

1. You use these pesticides for more than 6 days in a 30-day period, AND
2. The label of the pesticide(s) you use has the word "DANGER" or "WARNING" on it.

WHAT IS EXTRA MEDICAL CARE?

If you require extra medical care, you must get special blood tests so the doctor can tell if the pesticides are hurting your body. This may be happening even if you don't feel sick. The blood tests measure cholinesterase, a chemical in your body that helps your nerves work properly.

Organophosphates and carbamates can keep cholinesterase from working and harm your nerves. You must have the blood tests to make sure you aren't getting sick while working with these pesticides.

Having blood tests to check your cholinesterase **MUST** be part of your extra medical care.

SAFETY TIP

10

YOU MUST HAVE extra medical care if you often work with "Danger" or "Warning" organophosphate and carbamate pesticides.

CA Department of Pesticide Regulation • 1001 I Street, Sacramento CA 95814

PSIS No. A 10 (HS-8) 2018 A10 • 1

2. Medical Supervision Program Compliance Information

MEDICAL SUPERVISION FOR ORGANOPHOSPHATE AND CARBAMATE PESTICIDE HANDLERS

What employers need to know to comply with 3 CCR 6728

California Department of Pesticide Regulation

DPR Enforcement Branch

1001 I Street, P.O. Box 4015
Sacramento, CA 95812-4015
Tel.: (916) 324-4100
Fax: (916) 445-3907
www.cdpr.ca.gov

Northern Regional Office
West Sacramento
(916) 376-8960

Central Regional Office
Clovis
(559) 297-3511

Southern Regional Office
Anaheim
(714) 279-7690

Single copies of this handout are available from DPR by calling (916) 445-3974, or can be downloaded from DPR's website: www.cdpr.ca.gov, under "Fact Sheet Directory."

EMPLOYER RESPONSIBILITIES UNDER THE MEDICAL SUPERVISION PROGRAM

What is the Medical Supervision Program?

California's medical supervision program is required to protect agricultural workers who mix, load, or apply organophosphate or N-methyl carbamate pesticides with the signal word "DANGER" or "WARNING" on the label. If an employee works with such pesticides for more than six days in a consecutive 30-day period, blood levels of an important enzyme called cholinesterase must be monitored by a physician. If cholinesterase drops below certain levels, employers are required to take specific actions to prevent employee illness and injury.

What is cholinesterase and why monitor it?

Cholinesterase is important for normal function of the nervous system. Exposure to certain pesticides can inhibit this enzyme and cause illness. Common signs of overexposure include slow heart rate, difficulty breathing, salivation, tearing, sweating, abdominal pain, diarrhea, and confusion. Sometimes exposed individuals show no signs of illness. Monitoring serves to identify depressions in cholinesterase levels before an illness occurs.

How do I know which pesticides to look for?

Look for pesticides used to produce an agricultural commodity containing organophosphates or N-methyl carbamates with the signal word "DANGER" or "WARNING". Employers can identify these pesticides by looking at the "Precautionary Statements" and the "First Aid" sections on the product label.

When do I need an agreement with a physician?

You must have a physician monitor blood cholinesterase levels of employees who:

- mix, load or apply pesticides described here; *and*
- "regularly handle" such pesticides for more than six days in a consecutive 30-day period.

How do I find a physician to provide medical supervision?

Physicians must be registered with California's Office of Environmental Health Hazard Assessment to provide medical supervision. For a list of registered physicians see: https://oehha.ca.gov/pesticides/general-info/list-registered-medical-supervisors

How do I ensure my employees have a medical supervisor?

Employers must have a written agreement with a registered physician that:

- states the names and addresses of both the physician and the employer responsible for the employees;
- states the physician will provide medical supervision;
- states the physician is aware of and possesses a copy of the document "Medical Supervision of Pesticide Workers – Guidelines for Physicians" (available from the Office of Environmental Health Hazard Assessment website: goo.gl/DMLurG); *and*
- is signed by the physician.

This agreement must be filed by the employer with the county agricultural commissioner.

How often should my covered employees get blood cholinesterase levels tested?

Employees must get a baseline level established for both red blood cell cholinesterase and plasma cholinesterase. These are used to compare with subsequent cholinesterase tests and must be verified every two years.

Employees must have cholinesterase tests within three working days following each 30-day period when using pesticides described above for more than six days.

After three tests at 30-day qualifying periods, further monitoring is at intervals specified by the medical supervisor. If no written recommendation is given, the testing interval shall be 60 days.

ENSURE THE SAFETY OF YOUR EMPLOYEES!

Keep records detailing each day your employees handle (mix, load or apply) "Danger" or "Warning" organophosphates or carbamates to determine if your employee "regularly handles" these pesticides.

If you have employees who "regularly handle" these pesticides:
- have their blood cholinesterase levels tested; *and*
- have a written agreement with a registered medical supervisor.

Communicate with the medical supervisor to obtain:
- your employees' cholinesterase test results; *and*
- any occupational health recommendations.

Who will inform me of the cholinesterase test results and what do I do if they decline?

Your medical supervisor will notify you and your employee of the cholinesterase test results within 14 days of receiving them.

Employers are required to investigate the work practices of any employee whose red blood cell or plasma cholinesterase levels drop below 80% of baseline values. The investigation shall include review of the safety equipment used and its condition; and review of the employees sanitation, handling procedures, and equipment usage.

If cholinesterase levels fall to 70% or less of red blood cell baseline or 60% or less of plasma baseline, an employer must remove the employee from further exposure to organophosphate or carbamate pesticides until cholinesterase levels return to 80% or more of their respective baseline values.

What records must I keep under the medical supervision program?

- pesticide use records that identify the employee, name of the pesticide, and date of use;
- the written agreement with the medical supervisor;
- all recommendations received from the medical supervisor;
- all cholinesterase test results received from the medical supervisor;
- a written record of the work place investigation, findings, any changes made, and any recommendations given to the employee; *and*
- dates of employee removal and return to exposure, if employee is removed from exposure.

All records must be kept for three years.

IT'S THE LAW*

Physicians and other health care providers must report known or suspected pesticide-related illness and injury to the local health officer within 24 hours. The Office of Environmental Health Hazard Assessment is charged with providing training to physicians and other medical personnel on the recognition, treatment, and reporting of pesticide-related illness and injury.

More information: https://oehha.ca.gov/pesticides/education-and-training

* California Legislative Information: goo.gl/Uju81D

E HS-1760, 1/18

SUPERVISIÓN MÉDICA PARA MANIPULADORES DE PESTICIDAS DE ORGANOPHOSFATO Y CARBAMATO

Lo que los empleadores deben saber para cumplir con 3 CCR 6728

Departamento de Reglamentación de Pesticidas de California

DPR Enforcement Branch

1001 I Street, P.O. Box 4015

Sacramento, CA 95812-4015

Tel.: (916) 324-4100

Fax: (916) 445-3907

www.cdpr.ca.gov

Oficina Regional del Norte

West Sacramento

(916) 376-8960

Oficina Regional Central

Clovis

(559) 297-3511

Oficina Regional del Sur

Anaheim

(714) 279-7690

Copias de este folleto están disponibles en el DPR, llamando al (916) 445-3974, o pueden descargarla en el sitio web del DPR: www.cdpr.ca.gov, bajo " Fact Sheet Directory."

RESPONSABILIDADES DEL EMPLEADOR BAJO EL PROGRAMA DE SUPERVISIÓN MÉDICA

¿Qué es el Programa de Supervisión Médica?

Se requiere el programa de supervisión médica de California para proteger a los trabajadores agrícolas que mezclan, cargan o aplican pesticidas organofosforados o de carbamato de N-metilo con la palabra de advertencia "PELIGRO" o "AVISO" en la etiqueta. Si un empleado trabaja con dichos pesticidas durante más de seis días en un período consecutivo de 30 días, los niveles sanguíneos de una importante enzima llamada colinesterasa debe ser monitoreada por un médico. Si la colinesterasa cae por debajo de ciertos niveles, se requiere que los empleadores tomen medidas específicas para prevenir una enfermedad y daño del empleado.

¿Qué es la colinesterasa y por qué monitorearla?

La colinesterasa es importante para el funcionamiento normal del sistema nervioso. La exposición a ciertos pesticidas puede inhibir esta enzima y causar enfermedades. Los síntomas comunes de sobreexposición incluyen ritmo cardíaco lento, dificultad para respirar, salivación, lagrimeo, sudoración, dolor abdominal, diarrea y confusión. A veces, las personas expuestas no muestran señales de enfermedad. El monitoreo sirve para identificar las depresiones en los niveles de colinesterasa antes de que ocurra una enfermedad.

¿Cómo sé cuáles son estos pesticidas?

Busque los pesticidas utilizados para producir un producto agrícola que contenga organofosfatos o carbamatos de N-metilo con la palabra de advertencia "PELIGRO" o "AVISO". Los empleadores

pueden identificar estos pesticidas buscando en la etiqueta del producto las secciones "Declaraciones de Precaución" y "Primeros Auxilios".

¿Cuándo necesito tener un acuerdo con un médico?

Debe tener un médico que monitoree los niveles de colinesterasa en la sangre de los empleados que:

- mezclan, cargan o aplican pesticidas descritos aquí; y
- "que manipulen regularmente" tales pesticidas por más de seis días en un período consecutivo de 30 días.

¿Cómo puedo encontrar a un médico que brinde supervisión médica?

Los médicos deben estar registrados con la Oficina de Evaluación de Peligros a la Salud Ambiental de California para proporcionar supervisión médica. Para obtener una lista de médicos registrados, visite: https://oehha.ca.gov/pesticides/general-info/list-registered-medical-supervisors

¿Cómo me aseguro de que mis empleados tengan un supervisor médico?

Los empleadores deben tener un acuerdo por escrito con un médico registrado que:

- indique los nombres y direcciones del médico y del empleador responsable de los empleados;
- indique que el médico proporcionará supervisión médica;
- declara que el médico conoce y posee una copia del documento "Medical Supervision of Pesticide Workers – Guidelines for Physicians" [Supervisión Médica de Trabajadores con Pesticidas- Guías para Médicos] (disponible en el sitio web de la Oficina de Evaluación de Riesgos a la Salud Ambiental (Office of Environmental Health Hazard Assessment): goo.gl/DMLurG); y
- está firmado por el médico.

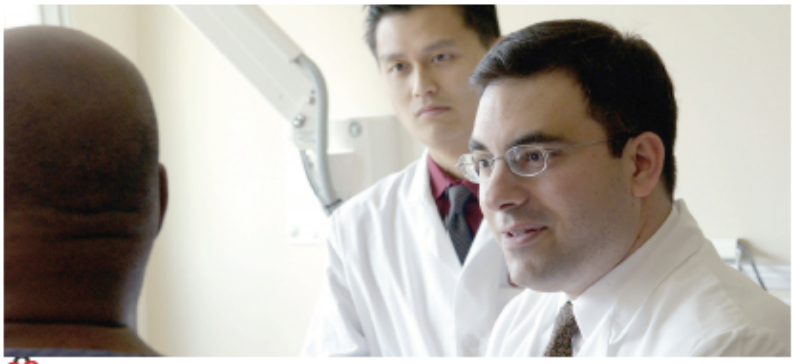
Este acuerdo debe ser presentado por el empleador con el Comisionado Agrícola del Condado.

¿Con qué frecuencia deben someterse a prueba mis empleados a los niveles de colinesterasa en la sangre?

Los empleados deben obtener un nivel de referencia establecido tanto para la colinesterasa de los glóbulos rojos como para la colinesterasa plasmática. Estos se usan para comparar con las pruebas posteriores de colinesterasa y se deben verificar cada dos años.

Los empleados deben someterse a pruebas de colinesterasa dentro de los tres días de trabajo posteriores a cada período de 30 días cuando usan los pesticidas descritos anteriormente durante más de seis días.

Después de tres pruebas en períodos de calificación de 30 días, se realiza un monitoreo adicional a intervalos especificados por el supervisor médico. Si no se proporciona una recomendación por escrito, el intervalo de prueba será de 60 días.



¡ASEGURE LA SEGURIDAD DE SUS EMPLEADOS!

Los empleadores deben tener un acuerdo por escrito con un médico registrado que:

- indique los nombres y direcciones del médico y del empleador responsable de los empleados;
- indique que el médico proporcionará supervisión médica;- declara que el médico conoce y posee una copia del documento "Medical Supervision of Pesticide Workers – Guidelines for Physicians" [Supervisión Médica de Trabajadores con Pesticidas- Guías para Médicos] (disponible en el sitio web de la Oficina de Evaluación de Riesgos a la Salud Ambiental (Office of Environmental Health Hazard Assessment): goo.gl/DMLurG); y
- está firmado por el médico.

¿Quién me informará sobre los resultados de la prueba de colinesterasa y qué debo hacer si no lo quieren hacer?

Su supervisor médico le notificará a usted y a su empleado sobre los resultados de la prueba de colinesterasa dentro de los 14 días después de recibirlo.

Se requiere que los empleadores investiguen las prácticas de trabajo de cualquier empleado cuyos niveles de colinesterasa en los glóbulos rojos o en el plasma caigan por debajo del 80% de los valores iniciales. La investigación incluirá un repaso sobre el equipo de protección utilizado y su condición; y un repaso del saneamiento de los empleados, procedimientos de la manipulación y el uso del equipo.

Si los niveles de colinesterasa caen al 70% o menos de línea base de glóbulos rojos o 60% o menos de línea de base plasmática, un empleador debe evitar al empleado de una mayor exposición a pesticidas organofosforados o carbamatos hasta que los niveles de colinesterasa vuelvan al 80% o más de sus valores basales respectivos.

¿Qué registros debo mantener bajo el programa de supervisión médica?

- registros de uso de pesticidas que identifican al empleado, nombre del pesticida y fecha de uso;
- el acuerdo por escrito con el supervisor médico;
- todas las recomendaciones recibidas del supervisor médico;
- todos los resultados de la prueba de colinesterasa recibidos del supervisor médico;
- un registro por escrito de la investigación del lugar de trabajo, hallazgos, cualquier cambio realizado y cualquier recomendación dada al empleado; y
- fechas de quitar al empleado y del regreso a la exposición, si el empleado fue retirado de la exposición.

Todos los registros deben conservarse durante tres años.

ES LA LEY*

Los médicos y otros proveedores de atención médica deben reportar conocidas o sospechosas enfermedades o lesiones relacionadas con los pesticidas al Oficial Local de la Salud dentro de las 24 horas. La Oficina de Evaluación de Riesgos a la Salud Ambiental se encarga de proporcionar capacitación a médicos y otro personal médico sobre el reconocimiento, tratamiento y reporte de enfermedades y lesiones relacionadas con pesticidas.

Más información: https://oehha.ca.gov/pesticides/education-and-training

* California Legislative Information: goo.gl/Uju81D

S HS-1760, 2/18

CALIFORNIA'S MEDICAL SUPERVISION PROGRAM

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

EMPLOYER FREQUENTLY ASKED QUESTIONS:

What is the Medical Supervision Program? California's medical supervision program is designed to protect the health of **agricultural workers** who mix, load or apply organophosphate or carbamate pesticides with signal word "Danger" or "Warning" on the label.

Who must participate in the Medical Supervision Program? Agricultural employers and their workers who handle such pesticides more than 6 days in a consecutive 30-day period participate in this program.

My employees have their cholinesterase levels regularly tested, and the medical supervisor is made aware of the employee's handling schedule. Have I met all my employer responsibilities? No. You must keep the medical supervision written agreement, all pesticide use records, recommendations from the medical supervisor, employee's cholinesterase test results and all employee records related to the program, for 3 years. You must ensure that the recommendations given by the medical supervisor, particularly, the frequency of an employee's cholinesterase tests are followed.

For employer/employee-related questions on the Medical Supervision Program, please contact:

Pesticide Illness Surveillance Program
Department of Pesticide Regulation
P.O. Box 4015, Sacramento, CA 95812-4015
pispillness@cdpr.ca.gov
(916) 445-4222

DPR's Regional Offices:
Northern, (West Sacramento): (916) 376-8960
Central, (Clovis): (559) 297-3511
Southern, (Anaheim): (714) 279-7692

The medical supervisor informed me that my employee's cholinesterase level is below 80% of baseline. What should I do? You must investigate the workplace practices, review the safety equipment, and its condition, as well as employee sanitation practices, and equipment usage. You must also maintain a written record of your findings, any changes in equipment or procedures, and any recommendations you gave your employee.

My employee's plasma cholinesterase is 60% or less of baseline, and/or RBC cholinesterase is 70% or less of baseline, but he or she does not feel sick. Is it okay to allow the worker to continue working with these pesticides? No. You must immediately remove your employee from further exposure to the pesticides until the depressed cholinesterase level (Plasma and/or RBC) is above 80% of his or her own baseline values. Keep a written record of the dates you removed an employee from further exposure, and dates an employee is returned to handling these pesticides. You should communicate with your medical supervisor to know when this employee is cleared to handle these pesticides.

What should I do before my newly-hired employee begins to mix, load or apply organophosphate and carbamate pesticides? You must have a written agreement with a physician to act as a medical supervisor. A copy of this document must be provided to the County Agricultural Commissioner no later than when your covered employee begins to regularly handle these pesticides. More importantly, before a new employee begins to handle these pesticides, you should send him/her to the medical supervisor to have his or her **baseline** cholinesterase level tested.

Can any physician act as a medical supervisor under this program? Any physician licensed to practice, who is aware of the contents of the Guidelines for Physicians from the Office of Environmental Health Hazard Assessment (OEHHA), and agrees to fulfill the requirements of this program, may be contracted as a medical supervisor. Since January 2017, physicians acting as medical supervisor are required to register with OEHHA.

Why is it important to monitor my employees' cholinesterase levels? Cholinesterase is an enzyme that helps in the normal function of the nervous system. Organophosphate and carbamate pesticides inhibit this enzyme from binding with a neurotransmitter. This can trigger symptoms of pesticide poisoning such as drooling, tearing, urination, loose bowel movements, upset stomach, vomiting, etc. In severe cases, this may lead to paralysis of the breathing muscle, causing death.

What do I tell the medical supervisor when I send my employees for cholinesterase testing? It is very important to inform the medical supervisor when your employee is newly-hired and needs a **baseline** cholinesterase test. Whenever your employee has began handling these pesticides, he or she should be sent for **follow-up** cholinesterase tests based on the medical supervisor's recommended frequency of testing. You also need to inform the medical supervisor whenever your employee has been a regular handler of these pesticides for 2 years, and needs a re-test to verify **baseline** cholinesterase level. Likewise, whenever you **suspect pesticide exposure** on your employee, it is crucial to let the medical supervisor know about it when you send your employee for medical attention.

My employees have been sent to the medical supervisor, and had their baseline cholinesterase determinations. What's next? You must follow the recommendations of the medical supervisor for your employees. It is also important to send your employee back for cholinesterase testing as often as indicated by the medical supervisor.

My employees handle these pesticides less than 6 days in a 30-day period. Should they be sent to the medical supervisor? The monitoring described in this bulletin is not required for these employees. However, <u>any</u> employee exposed to these pesticides, or reports any symptoms described above, should immediately be sent for medical attention.

Should my employees be able to readily contact the medical supervisor? Definitely. The name, address and telephone number of the medical supervisor must be posted at a place easily seen by your employees at the beginning of each work day.

Appendix C: PCB Survey and Inspection Project

1. Project Protocol and Findings

I. **Project Title:** Agricultural Pest Control Businesses Survey for Knowledge of and Compliance with the Medical Supervision Program

II. **Survey Purpose:** To collect information from pest control businesses (PCBs) on their knowledge of and compliance with the Medical Supervision Program's ("Program") requirements. Information obtained from this survey will supplement analysis of the cholinesterase (ChE) test results reported by diagnostic laboratories, and will be used in the next evaluation of the Program.

III. **Methods:** Using 2014–2016 data from DPR's Pesticide Use Report and the ChE test results records reported by the laboratories to DPR, Worker Health and Safety-Pesticide Illness Surveillance Program (PISP) and Enforcement (ENF) Branch staff identified 3–4 counties from each of DPR's three regional offices in which to conduct the surveys and inspections. Ten counties with the highest amount of organophosphate (OP) and carbamates (CB) pesticides applied, and the highest count of ChE test results received by DPR for that same year range were selected: Fresno, Kern, Monterey, Colusa, San Joaquin, Yolo, Imperial, Riverside, Santa Barbara and Ventura. Within each of these counties, three PCBs that showed a high use of OP/CB were randomly selected, with an additional 1–2 PCBs that served as alternates for survey and inspection.

PISP staff developed a questionnaire to collect data on the following key areas of the Program (See Appendix C2):

- Whether an employer with employees in the Program kept a record of the medical supervisor's (MS) written agreement, including all recommendations received from the MS, and all results of their employee's ChE tests for the last three years.
- Whether an employer with employees in the Program gave a copy of the MS written agreement to the County Agricultural Commissioner's (CAC) office no later than when an employee began to regularly handle pesticides.
- Whether an employer with employees in the Program investigated the work practices of any employee whose red blood cell (RBC) ChE or plasma ChE levels fell below 80% of baseline.
- Whether an employer with employees in the Program removed the employee from exposure to organophosphate or carbamate pesticides if the employee's plasma ChE level fell to 60% or less of baseline, or if RBC ChE fell to 70% or less of baseline.

IV. **Results:** Of the 50 PCBs selected for this project, the ENF Branch staff surveyed a total of 42 PCBs. Seventeen of these PCBs were from DPR's central region, 10 were from the northern region, while 15 were from the southern region. Of the 42 PCBs, only 21 had employees who regularly handled OPs/CBs more than 6 days in a given 30 consecutive days. Ten of the 21 PCBs were from the southern region, nine from the central region, and two were from the northern region (Figure C1a). These employees were covered under the Program and required medical supervision.

- **Medical Supervision Written Agreement.** Of the 21 PCBs with employees under the Program, 17 had copies of their written medical supervision agreement filed with their respective CACs. Four had no copy of their written agreement on file with the CAC. Of those four PCBs, one dropped their medical supervisor after 2015 because they stopped using OP/CB; one had an expired medical supervision agreement; one had an agreement not signed by the MS; and one PCB did not specify a reason why there was no copy of their written agreement with the CAC.

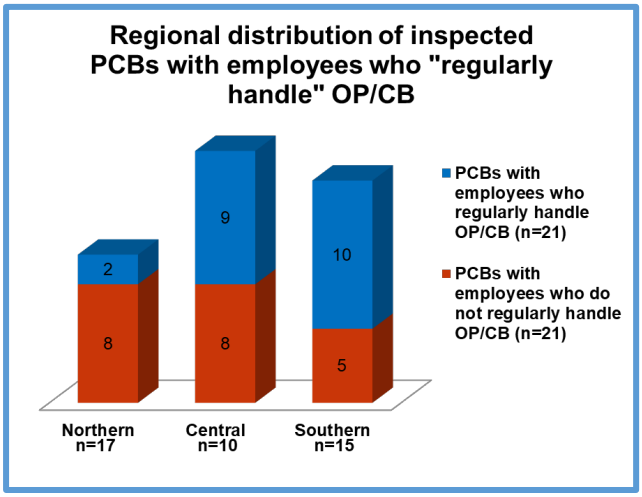


Figure C1a: *Number of PCBs with employees who handle OP/CB by region.*

- **ChE Level Monitoring.** Six of the 21 PCBs indicated that their MS determined the frequency of the ChE level monitoring of their employees who regularly handled OP/CB. One of these six MS recommended the ChE testing of covered employees every 365 days. Ten PCBs indicated that their MS did not provide recommendations for ChE testing intervals, so they (the PCB employer or owner themselves) determined the frequency for testing of their employees under the Program. Five of the PCBs did not send their employees who regularly handled OP/CB for periodic monitoring or follow-up, which was not in compliance with regulation.

- **Investigate Work Practices**. Of the 21 PCBs, three employers acknowledged investigating employees' work practices because their ChE (plasma or RBC) test results fell below 80%. Of those three, two PCBs received recommendations from the MS to investigate work practices of the employee whose plasma or RBC test results fell below 80%.

- **Removal of Employee.** Two PCBs had to remove an employee when his or her RBC ChE fell to 70% or lower, or plasma ChE fell to 60% or lower. Of these two, one PCB received a recommendation from the MS to "remove an employee", while the other did not. Another PCB responded that the MS recommended removal of an employee, even though the ChE test result was higher than the regulation's removal threshold (*ChE RBC of 70% or less of baseline, ChE plasma of 60% or less of baseline*). This physician took over as a MS midway through this project, and was not registered with OEHHA (See Discussion).

- **Records Retention.** The Program requires employers with employees who regularly handle OP/CB to keep copies of the following for 3 years: (1) pesticide use records, (2) the medical supervision agreement, (3) all ChE test

results, and (4) recommendations from the MS for the tested employee (Table C1a).

Table C1a: *Records retentions in accordance with 3 CCR § 6728(3); n=21.*

Record Type	Yes, Kept Records	No, Did NOT Keep Records	NA	No Answer
Pesticide Use Records	13	7	0	1
Copy of MS Agreement	18	2	0	1
Recommendation from MS	7	4	9	1
Employee ChE test Result	20	0	0	1

- **Emergency Medical Care** (3 CCR § 6726) in particular is not part of the Program. Nonetheless, this question was included in the survey to ascertain what action employers took when a handler was ill or had a suspected exposure to OP/CB. The following were the responses from the 21 PCBs:
  - Seven indicated that they sent their employees for immediate medical care, two of the seven to their MS.
  - Nine indicated that none of their OP/CB handlers got exposed or reported illness from these pesticides
  - Five PCBs did not provide responses

- **"Purpose" of test indicated when ordering ChE Tests.** The MS relies on the employer to indicate the "*purpose*" of the ChE test (such as "baseline," "follow-up," "illness or exposure") whenever an employee is sent for ChE level testing (Table C1b).

Table C1b: *Proportion of PCBs that informed their medical supervisors of the "Purpose" of test when an employee was sent for ChE Testing.*

Purpose of ChE Test	Informed MS	Did not Inform MS	NA	Total PCB
Baseline	20	1	0	21
Follow-Up	15	4	2	21
"Illness or "Exposure"	7	7	7	21

Almost all 21 PCBs informed the MS when an employee needed "baseline" ChE tests, before these covered employees began handling OP/CB. About three quarters informed their MS when an employee handled OP/CB > 6 days ("follow-up"), and a third when the employee was "ill" or "exposed" to OP/CB. Only one PCB did not inform the MS if an employee needed "baseline" ChE tests, while four did not inform the MS when an employee handled OP/CB more than 6 days in a given 30-day period, and required ChE level monitoring ("follow-up"). A third of these PCBs did not inform the MS when an employee had an "illness" from or "exposure" to OP/CB. Some of the responses were:

- One did not keep records
- One did not know the Program requirements

- One used only a closed system
- Four provided no reason

- **Provide Copy of the ChE Test Results.** Of the 21 PCBs, 20 confirmed receiving their employee's ChE test results. However, only 11 relayed the test results to the tested employee (Table C1c).

Table C1c: *Medical supervisor provided ChE test results according to HSC § 105206.*

MS Provided ChE Test Results	Yes	No	Not Applicable	No Answer	Total
Employer **received** ChE test results of employee	20	0	0	1	21
Employer **relayed** ChE test results to employee	11	9	0	1	21

V. Discussion: Eighteen of the 21 PCBs whose employees were covered under the Program had a written agreement with an MS. Of these 18 MS, over a quarter (5) were not registered with OEHHA. DPR forwarded to OEHHA the names of these five unregistered MS. Despite the fact that only one of the 18 MS indicated a ChE level monitoring of covered employees that is greater than every 60 days, that particular medical supervisor's registration with OEHHA is current. Of the three that did not have a written agreement, two provided a health care facility name, but not a specific physician.

Two thirds (14) of the 21 PCBs were able to define "regularly handling" in their own words, however, a third (7) of these PCBs could not correctly state the criteria for "regularly handling".

While the majority of the 21 PCBs whose employees required medical supervision kept pesticide use records, a third gave the following reasons for not meeting specific Program requirements:

- Three could not locate pesticide use records, or kept these offsite
- Two ended their medical supervision agreement after 2015
- One did not know that ChE testing needs to be performed on employees who regularly handled OP/CB
- One did not keep ChE test results or employee records of exposure or illness

Overall, the 21 PCBs surveyed that had employees who regularly handled OP/CB were aware of the Program, but had varying degrees of understanding of its specific requirements. Almost two thirds (13) of these PCBs knew to keep pesticide use records, and almost all (18) kept a copy of the written medical supervision agreement. Finally, three quarters (16) of these PCBs have their employees covered under the Program sent for periodic ChE levels testing in compliance with regulation, while only one of these PCBs did not know to send an employee who regularly handles OP/CB for the periodic 60-day frequency of ChE level testing when no recommendation was received from their contracted MS.

2. Questionnaire for PCB Medical Supervision Project

Questionnaire for Pest Control Business (PCB) Medical Supervision Project

Business Name and License: _____ Date of Inspection: _____
Business Address: _____ Tel: _____
Contact Person: _____ Title: _____
Medical Supervisor Name (MS): _____ MD/DO/NP/PA
MS Affiliation: _____ Tel: _____
Affiliation Address: _____
Copy of MS agreement at CAC's office: ☐**Yes** ☐**No**

Survey Questions

1. What was the total number of employees (from 2014-2016) who handled Organophosphates (OPs) and Carbamates (CBs)?	#		
2. Can the employer define "regularly handle" (3CCR § 6000) in his/her own words?	☐Yes	☐No	
3. Did employer have employees (from 2014-2016) who **regularly handled** (3CCR § 6000) OP/CB pesticides with signal word "Danger" or "Warning?"	☐Yes	☐No **Go to 3a**	☐N/A
How many employees (from 2014-2016) **regularly handled** OPs/CBs?	#		
3a. ***If NO***, what steps were taken to ensure that employees did not use OPs/CBs for more than 6 days in any 30-day period? ☐ Handlers were given other duties prior to 6 days in a 30-day period ☐ Employer no longer used OPs/CBs → Since when? _____ ☐ Other reason, please specify:_____			
4. Did employer send employee(s) who regularly handle OPs/CBs for cholinesterase (ChE) level testing? (3CCR § 6728 (c)(1)(2))	☐Yes	☐No **Go to 4c**	☐N/A
4a. ***If YES***, how often was the OP/CB regular handler sent for routine testing? Every: ☐ 30 days ☐ 60 days ☐ 365 days ☐ Other, please specify:_____ ☐ Don't know			
4b. If yes, who determined the schedule/frequency of employee's routine testing.	☐MS	☐Employer	☐N/A
4c. ***If NO***, what was the reason? ☐ Handlers were given other duties prior to 6 days in a 30-day period ☐ Employer did not have a medical supervisor ☐ Employer not aware of this requirement ☐ Other reason, please specify:_____			
5. When an employee(s) was ill from or may have been exposed to OPs/CBs, the employer: ☐ Sent the employee for immediate medical care ☐ Sent employee(s) to MS ☐ Was/Is not aware of this requirement ☐ Other, please specify:_____			
6. When employer sent an employee to an MS for testing, did employer inform the MS if:			
6a. Employee needed a baseline established? (3CCR § 6728 (c)(1))	☐Yes	☐No	☐N/A
6b. Employee handled OPs/CBs >6 days in a 30-day period? (3CCR § 6728 (c))	☐Yes	☐No	☐N/A
6c. There was any suspicion or actual OP/CB exposure or illness?	☐Yes	☐No	☐N/A

PCB Survey and Inspection Protocol for Medical Supervision Page **1** of **2**

7. Did the employer receive the ChE test results of the employee? (3CCR § 6728 (c)(3))	☐Yes	☐No	☐N/A
8. Did the employer relay the ChE test results to the tested employee? (3CCR § 6728(c)(3))	☐Yes	☐No	☐N/A
9. Did the employer investigate work practices when employee's ChE test result was below 80% of baseline? (3CCR § 6728 (d))	☐Yes	☐No	☐N/A
9a. Did the MS recommend to investigate work practices?	☐Yes	☐No	☐N/A
10. Did the employer remove an employee from handling OPs/CBs when RBC ChE baseline fell to 70% or less, and/or plasma ChE baseline fell to 60% or less? (3CCR § 6728 (e))	☐Yes	☐No	☐N/A
10a. Did the MS recommend removal of employee from handling OPs/CBs?	☐Yes	☐No	☐N/A
11. Records retention (from 2014-2016):			
11a. OP/CB use records? (3CCR § 6728 (c)(3))	☐Yes	☐No	☐N/A
11b. Copy of MS Agreement at PCB's office?	☐Yes	☐No	☐N/A
11c. Recommendations from MS? (3CCR § 6728 (c)(3))	☐Yes	☐No	☐N/A
11d. Employee's ChE test results? (3CCR § 6728 (c)(3)) ***If YES***, in the form of: ☐ Actual ChE test results value? ☐ Summary/narrative of ChE test results ***If NO***, why? ☐ Have not received any ☐ Other, please specify:_____	☐Yes	☐No	☐N/A

Complete Appendix C3: Employee Information Table for all OP/CB regular handlers.
Comments

3. PCB Employee Information Table

Employee Information Table (Regular Handlers of OPs/CBs), Survey

BUSINESS NAME & LICENSE: INSPECTOR: DATE OF SURVEY/INSPECTION:

(2014-2016) Employee Name (First and Last Name)	AGE (DOB, if available)	START DATE Handled OP/CB (2014-2016)	Received Copy of CHE TEST RESULTS from?	Received Copy of MS RECOMMD'N from?	DATE Workplace INVESTIG'D, If applicable	REMOVED from Handling OP/CB?	DATE(S) REMOVED from Handling OP/CB	DATE(S) RETURNED to Handling OP/CB, If applicable	Other Reason(s) 'REMOVED' (Specify)
			☐MS ☐Employer ☐None ☐N/A	☐MS ☐Employer ☐None ☐N/A	☐No MS Recommd'n	☐Yes ☐No	☐No MS Recommd'n	☐No MS Recommd'n	
			☐MS ☐Employer ☐None ☐N/A	☐MS ☐Employer ☐None ☐N/A	☐No MS Recommd'n	☐Yes ☐No	☐No MS Recommd'n	☐No MS Recommd'n	
			☐MS ☐Employer ☐None ☐N/A	☐MS ☐Employer ☐None ☐N/A	☐No MS Recommd'n	☐Yes ☐No	☐No MS Recommd'n	☐No MS Recommd'n	
			☐MS ☐Employer ☐None ☐N/A	☐MS ☐Employer ☐None ☐N/A	☐No MS Recommd'n	☐Yes ☐No	☐No MS Recommd'n	☐No MS Recommd'n	
			☐MS ☐Employer ☐None ☐N/A	☐MS ☐Employer ☐None ☐N/A	☐No MS Recommd'n	☐Yes ☐No	☐No MS Recommd'n	☐No MS Recommd'n	
			☐MS ☐Employer ☐None ☐N/A	☐MS ☐Employer ☐None ☐N/A	☐No MS Recommd'n	☐Yes ☐No	☐No MS Recommd'n	☐No MS Recommd'n	
			☐MS ☐Employer ☐None ☐N/A	☐MS ☐Employer ☐None ☐N/A	☐No MS Recommd'n	☐Yes ☐No	☐No MS Recommd'n	☐No MS Recommd'n	

Appendix D: OEHHA Literature Review

BACKGROUND

Organophosphate (OP) and carbamate (CB) pesticides are widely used in agriculture and human health effects from OP and CB exposure have been well documented (Coye, 1986). Despite significant reductions in the number and amount of OP and CB pesticides used in recent decades, pesticide handlers are still often routinely exposed to high levels of these hazardous ChE-inhibiting pesticides. While personal protection equipment and engineering controls can reduce a worker's exposure to harmful chemicals, routine surveillance of ChE activity levels can offer further protection to vulnerable workers from excessive exposure and thereby reduce pesticide-related illnesses.

Introduced in 1974, the California Medical Supervision Program (hereafter, the Program) is a biomonitoring program that has been established to identify and prevent excessive OP and CB exposure in pesticide handlers and protect high-risk workers from pesticide-related illness. The Program requires that agricultural workers who regularly handle OP and CB pesticides in Toxicity Categories I and II—pesticides that are labelled with the words "DANGER-POISON" or "WARNING" – have their blood ChE activity levels monitored by physicians registered with the California Environmental Protection Agency.

The primary mechanism of toxicity of OP and CB pesticides is the inhibition of cholinesterase (ChE), an enzyme in the neuromuscular junction and peripheral nerves of the brain (Dyer, 2001; Krenz, 2015; Strelitz, 2014). By preventing the breakdown of the neurotransmitter acetylcholine, OP and CB pesticides cause both immediate neurotoxic effects and delayed neurodegenerative disease. Plasma ChE (or butyrylcholinesterase) and RBC ChE (or acetylcholinesterase, AChE) are two enzymes found in the blood that are commonly used as biomarkers of the AChE found in the nervous system. Since different pesticides preferentially bind to the RBC or plasma ChE enzyme, ChE surveillance programs recommend testing for both RBC ChE and plasma ChE.

There is substantial inter-individual variation in ChE activity within the general population (Brock, 1991; Mason, 2000) due to a number of variables (e.g., genetic variation, lifestyle habits, medication etc.). Thus, a reference ChE baseline measurement for each individual is often necessary to accurately estimate the degree of ChE inhibition (Coye, 1987). Intra-individual variation in ChE activity has also been documented (Brock, 1990; Brock, 1991: Lefkowitz, 2007). Thus, regular verification of baseline levels is often recommended in illness surveillance programs.

To assess the usefulness of the Program's requirements for the purpose of surveillance, the Office of Environmental health Hazard Assessment (OEHHA) conducted three literature reviews. The first systematic literature review identified epidemiological studies linking exposure to OP and/or CB pesticides and ChE activity levels to find evidence that workers' exposure to ChE-inhibiting pesticides induce significant levels of ChE activity inhibition and therefore validate the use of ChE as a biomarker of exposure. The second systematic literature review identified epidemiological studies linking ChE activity depressions due to exposure to OP and/or CB pesticides to adverse health outcomes in order to validate the use of ChE levels as a biomarker of effect. And finally, the third literature review examined intra and inter individual variations of both RBC and plasma ChE activity levels in the population of working adults with no known exposures to ChE-inhibiting chemicals for the purpose of validating the requisite for regular baseline levels to which follow-up must be compared to. This review also explores the physiological evidence for such variations.

1. Literature Review on Cholinesterase Activity Levels and Health Effects in Workers Exposed to OP/CB Pesticides

OBJECTIVE

The objective of this review is to find evidence that workers' exposure to ChE-inhibiting pesticides induce significant levels of ChE activity inhibition and therefore validate the use of ChE as a biomarker of exposure. For this, we examined the existing peer-reviewed literature linking exposure to OP and/or CB pesticides to ChE activity levels.

METHODS

PECO Statement

We created a Population, Exposures, Comparator and Outcome (PECO) statement (Vandenberg et al., 2016), a modified version of Cochrane's PICO principle, to guide our search.

Element	Explanation
Population (P)	Human subjects only, occupational setting only
Exposure (E)	Cholinesterase inhibiting chemicals such as organophosphates and carbamates
Comparator (C)	ChE activity levels in the blood both RBC and plasma ChE
Outcome (O)	Symptoms such as dizziness, visual problems, headache, nausea, vomiting, diarrhea and other GI symptoms, and respiratory symptoms.

Search Strategy

The PubMed research database (https://www.ncbi.nlm.nih.gov/pubmed/) was used to identify relevant studies. The PubMed search was done by OEHHA's librarian on December 18, 2019 and included articles published until then. The search was limited to full articles available in English. Search terms are presented in Table D1a. The search strategy involved using a combination of four domains. In the first domain there were population terms such as occupational settings. The second domain contained comparator terms affiliated to ChE activity. The third domain consisted of exposure terms, which are pesticides and other ChE-inhibiting chemicals. Finally, in the fourth domain contained outcome terms related adverse health effects and symptoms.

PubMed Search Terms

Table D1a: *PubMed literature search on December 2018, 2019.*

Steps	Search Terms	Number of Citations	Concepts
1	cholinesterase inhibitors/toxicity[mh] OR cholinesterase inhibitors/poisoning[mh] OR organophosphates/adverse effects[mh] OR organophosphates/poisoning[mh] OR organophosphates/toxicity[mh] OR organophosphates/blood[mh] OR "organophosphorus compounds"[mh] OR "organophosphate poisoning"[mh] OR carbamates/poisoning[mh] OR carbamates/toxicity[mh] OR cholinesterases/adverse effects[mh] OR cholinesterase inhibitor*[tiab] OR carbamate*[tiab] OR organophosphorus[tiab] OR organophosphate*[tiab] OR cholinesterase[tiab] OR anticholinesterase[tiab] OR anti-cholinesterase[tiab] OR acetylcholinesterase inhibit*[tiab] OR ache inhibit*[tiab] OR Butyrylcholinesterase inhibit*[tiab] OR Plasma ChE inhibit*[tiab]	189957	cholinesterase inhibitor concept
2	pesticides/poisoning[mh] OR pesticides/toxicity[mh] OR "Pesticides" [Pharmacological Action] OR pesticide*[tiab] OR insecticide*[tiab] OR herbicide*[tiab] OR insect repellent*[tiab] OR carbaryl OR dichlorvos OR chlorpyrifos OR fenitrothion OR carbofuran OR fenthion	192113	pesticide terms
3	("cholinesterase activity"[tiab] OR "acetylcholinesterase activity"[tiab] OR "acetyl cholinesterase activity"[tiab] OR "ache activity"[tiab] OR Butyrylcholinesterase activity[tiab] OR "Butyryl cholinesterase activity"[tiab] OR Plasma ChE activity[tiab] OR cholinesterase level*[tiab] OR acetylcholinesterase level*[tiab] OR ache level*[tiab] OR Butyrylcholinesterase level*[tiab] OR Plasma ChE level*[tiab] OR "blood cholinesterase"[tiab] OR “blood che”[tiab] OR "blood acetylcholinesterase"[tiab] OR "blood acetyl cholinesterase"[tiab] OR "blood ache"[tiab] OR blood Butyrylcholinesterase[tiab] OR “blood Butyryl cholinesterase”[tiab] OR blood plasma ChE[tiab] OR "serum cholinesterase"[tiab] OR “serum che”[tiab] OR "serum acetylcholinesterase"[tiab] OR "serum acetyl cholinesterase"[tiab] OR "serum ache"[tiab] OR serum Butyrylcholinesterase[tiab] OR Butyryl cholinesterase[tiab] OR serum plasma ChE[tiab])	12814	cholinesterase activity terms
4	(“signs and symptoms”[mh] OR symptom*[tiab] OR adverse effect*[tiab] OR "adverse health effects"[tiab] OR clinical[tiab] OR hematologic[tiab] OR dysfunction*[tiab] OR impair*[tiab] OR symptom*[tiab] OR depression[tiab] OR neurologic*[tiab] OR neurodegenerat*[tiab] OR respiratory[tiab] OR cardiac[tiab] OR heart[tiab] OR cardiovascular[tiab] OR metabolic[tiab] OR reproducti*[tiab] OR autoimmune[tiab] OR diaphoresis[tiab] OR perspiration[tiab] OR sweating[tiab] OR gastrointestinal[tiab] OR diarrhea[tiab] OR bradycardia[tiab] OR bronchospasm[tiab] OR bronchorrhea[tiab] OR emesis[tiab] OR vomiting[tiab] OR lethargy[tiab] OR lacrimation[tiab] OR salivation[tiab] OR headache[tiab] OR fatigue[tiab] OR paresthesia*[tiab] OR numbness[tiab] OR weakness[tiab] OR nausea[tiab] OR dizziness[tiab] OR syncope[tiab])	8561368	adverse effects and clinical symptoms terms

Steps	Search Terms	Number of Citations	Concepts
5	#3 OR #4	8569868	RBC ChE activity OR Symptoms
6	#1 AND #2 AND #5	7992	combine above for most inclusive results
7	(animals[mh] NOT humans[mh])	4650959	animal terms
8	(Rat[ti] OR rats[ti] OR mouse[ti] OR model[ti] OR mice[ti] OR pig[ti] OR pigs[ti] OR murine[ti] OR porcine[ti] OR dog[ti] OR dogs[ti] OR canine[ti] OR monkey[ti] OR primate[ti] OR rabbit[ti] OR rodent[ti] OR animal[ti] OR ovine[ti] OR xenograft[ti] OR baboon[ti] OR horse[ti] OR equine[ti] OR gerbil[ti] OR hamster[ti] OR guinea pig[ti] OR zebra[ti] OR lamb[ti] OR calf[ti] OR calves[ti] OR macaque*[ti] OR swine[ti] OR Wistar[ti] OR ram[ti] OR rams[ti] OR sheep[ti] OR hamster[ti] OR marsupial[ti] OR dunnart[ti] OR Sprague Dawley[ti] OR mare[ti] OR mares[ti] OR ewe[ti] OR ewes[ti] OR piglet*[ti] OR zebrafish[ti] OR fish[ti] OR trout[ti] OR minipig*[ti] OR snail[ti] OR bird[ti] OR birds[ti])	2386580	animal terms
9	#7 OR #8	5254315	combine animal terms
10	review[pt] OR meta-analysis[pt] OR meta-analysis[tiab] OR metaanalysis[tiab] OR systematic[sb]	2704191	reviews & meta-analysis
11	#6 NOT (#9 OR #10)	3044	remove animals, reviews, meta-analysis
12	occupational exposure[mh] OR farmers[mh] OR occupational[tiab] OR job exposure*[tiab] OR worker*[tiab] OR farmer*[tiab] OR farmworker*[tiab] OR farming[tiab] OR farm[tiab] OR farms[tiab] OR farmhand*[tiab] OR field hand*[tiab] OR greenhouse*[tiab] OR nursery[tiab] OR garden*[tiab] OR landscap*[tiab] OR horticultur*[tiab] OR handler*[tiab] OR applicator*[tiab] OR sprayer*[tiab]	458674	occupational exposure
13	#11 AND #12	770	FINAL with occupational exposure concept

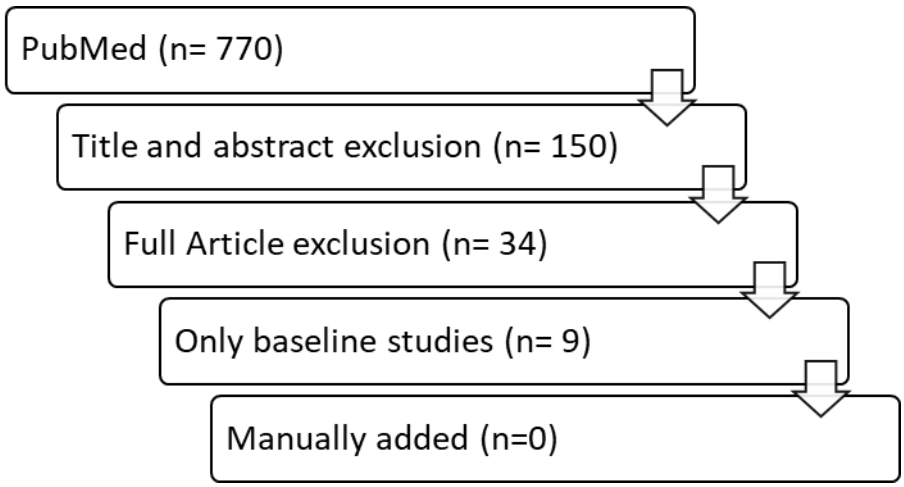
Study Selection

Table D1b: *Exclusion criteria.*

Exclusion Criteria
1. Reviews, editorials, methodological articles, and meta-analyses
2. Not in English
3. Titles with no text
4. Animal studies
5. No ChE activity
6. No adverse health effects or no symptoms
7. Not occupational exposure
8. No correlation between ChE levels and symptoms

The initial phase of this process was to omit irrelevant articles by title and abstract analysis. The second phase was to omit articles based on their full content (Table D1b). The third phase was to only select articles for which baseline levels were available. Finally, frequently cited studies in the literature that were not in our literature pool were considered to be manually added.

Table D1c: *Exclusion criteria flow chart.*



Total n = 9

Critical Appraisal of Studies

Criteria for the nine studies finally selected for this review were:

- They had to have baseline levels of RBC ChE and plasma ChE
- Exposure to ChE-inhibiting pesticides
- Post-exposure levels of RBC and plasma ChE for their subjects
- The subjects had to have signs and symptoms that developed as a result of their exposures that were consistent with those of ChE inhibition.

Ultimately, only nine studies of the 770 results of the PubMed search were found relevant and useful in our literature search (Table D1c).

RESULTS

All nine studies looked at the effects of exposure of workers to ChE-inhibiting pesticides and had values for RBC and/or plasma ChE for each subject (Table D1d). The ChE values taken when there was little or no exposure served as baselines to which post-exposure RBC and plasma ChE were compared. This allowed for the calculation of the amount of inhibition of RBC and plasma ChE activity levels as a percentage of the baselines. These studies also noted health effects that resulted from exposure to ChE-inhibiting pesticides, which could be correlated to the percentage of RBC and plasma ChE inhibition that occurred. The nine studies included in this review were of several study designs.

There were 893 subjects in these nine studies who were all workers occupationally exposed to mainly Toxicity Categories I and II OP and/or CB pesticides. The studies in Table D1d were separated into three groups:

- Workers who applied agricultural pesticides (five studies)
- Fieldworkers and indoor harvesters (one study of three different incidents
- Pesticide plant workers (three studies)

All the studies looked at the health effects that developed in these workers and the levels of inhibition of RBC and/or plasma ChE associated with those effects. These studies had baseline pre-exposure ChE levels to which post-exposure ChE levels were compared except for the study of fieldworkers and indoor harvesters who did not have pre-exposure baselines but had post-exposure serial tests (Coye et al, 1987). Their post-exposure ChE values after recovery were used as their baselines.

The most common symptoms reported in these studies were dizziness, visual problems, headache, nausea, vomiting, and other gastrointestinal symptoms, respiratory symptoms, and fatigue. Other symptoms and signs mentioned included muscle fasciculation, cramp, diaphoresis, numbness, chest pain, salivation, and lacrimation. None of the signs and symptoms would be pathognomonic for ChE inhibition, but they can all be seen and consistent with it.

In the nine studies, the range of inhibition of RBC ChE was 0-87%. The negative inhibition of 10% in Kashyap et al. (1984) was considered to be a 0% inhibition in this review. The range of inhibition of plasma ChE was 9-97%. Two of the studies measured only RBC ChE (Leng et al, 1999 and Smit et al, 2003) and one measured only plasma ChE (Khan et al, 2010). The studies were separated into three groups because of the similarities of their occupations and exposures.

Table D1d: *Summary of all nine studies included in this review.*

Study	Subjects*	Pesticides	Symptoms (and Signs)*	ChE Analyzed & % Inhibition from Baseline	Comments*
Subjects: Agricultural Pesticide Applicators					
Fillmore, C. et al., (1993)	79 pesticide handlers in the Program in San Joaquin Valley had ongoing monitoring. 5 workers with symptoms had Plasma ChE <60% of baseline.	Parathion Methidathion Carbophenthion Mevinphos Chlorpyrifos Dimethoate Naled EPBP Phosalone Formentanate Methomyl Carbaryl	Blurred vision, double vision, lacrimation, salivation, hand, arm, and feet numbness, CP, diarrhea, SOB, nervousness, dizziness, rash	Plasma ChE: 42-87% RBC ChE: 0-8%(Range for the 5 subjects)	Retrospective study of a medical supervisor's monitored workers in the Program in 1989 and 1990. 155 workers had baselines. 79 had ongoing monitoring. 24 had ongoing monitoring for both years. 5 workers with symptoms had Plasma ChE <60% of baseline.
			Reported by HCP	Ellman Method	
Jintana, S. et al, (2009)	Exposed group was 90 pesticide applicators in Thailand. Control group was 30 non-exposed volunteers	13 OPs Most common were: Dichlorvos (25%) Chlorpyrifos (20%) Dimethoate (17%)	Dizziness in 88% Headache In 91% Nausea in 82%	<u>Using baseline</u>: RBC ChE: 30% Plasma ChE: 26% (means) <u>Using controls</u>: RBC ChE: 47% Plasma ChE: 37% (means)	ChE and symptoms information collected during a period of low application of pesticides and used as baselines. Follow-up was done 3 mos. later during a period of high application of pesticides.
				Ellman Method	

Study	Subjects*	Pesticides	Symptoms (and Signs)*	ChE Analyzed & % Inhibition from Baseline	Comments*
Khan, D. et al, (2010)	105 tobacco farmers who applied pesticides ≥30 hrs/mos. in Pakistan	Methomyl Thiodicarb Methamidophos Occasionally: Pyrethroids Organochlorines	HA, N, V, SOB, dizziness, skin rash, eye redness, muscle weakness	Plasma ChE: 30-38% (Range)	105 tobacco farmers in study. A medical doctor took the history and did exams pre-exposure and 1-4 hrs after spraying on different days during the study period. Plasma ChE were done at beginning of crop season, Mar-Apr 2007 post spray in May-June 2007.
			Reported by HCP	Ellman Method	
Pathak, M. et al, (2013)	18 agricultural pesticide sprayers in Northern India	Chlorpyrifos Monocrotophos Dichlorvos Malathion Methyl parathion Quinalphos Carbendazim Cypermethrin Sulfur	Weakness of arms and legs, itching eyes, body ache, HA, dizziness. FEV1 decreased 20%	RBC ChE: 55% (sig) Plasma ChE: 9% (not sig) (means) RBC ChE Post=249.02±85.34 Pre=551.62±235.99 Post/Pre=45% (p<0.001) Plasma ChE Post=254.59±120.61 Pre=279.21±232.84 (not sig)	Data was taken before and after pesticide spraying season (4 mos.). Post exposure data obtained within 1 week after spraying. ChE, spirometry, NCV, and symptoms were studied. No change in NCV was found.
				Ellman Method	
–Smit, L., et al, (2003)	216 farmers (94 general farmers and 122 IPM) in Sri Lanka who applied pesticides	Chlorpyrifos most used. Subjects could not remember the names of other pesticides	Fainting, unconsciousness, HA, N, V, dizziness, blurred vision	General farmers RBC ChE: 10.58% (SD=7.19) (means) IPM farmers RBC ChE: 8% (SD=6.22) (means) Control RBC ChE: 3.2% (SD=5.89)	RBC ChE and data obtained in 2 periods: Apr-May 2000 (low exp., used as baseline) and June-July 2000 (high exp.). IPM farmers reported considerably less pesticide use. Control group of 40 fishermen did not add any info.
				Ellman Method	

Study	Subjects*	Pesticides	Symptoms (and Signs)*	ChE Analyzed & % Inhibition from Baseline	Comments*
Subjects: Harvest Workers					
Coye, M. et al, (1987)	I. 16 field workers II. 29 lettuce harvesters in Salinas CA. III. 18 mushroom farm workers Salinas CA	I. Mevinphos Phosphamidon II. Mevinphos III. Diazinon	Blurred vision, eye irritation, HA, N, V, diarrhea, CP, SOB, fatigue, dizziness, diaphoresis, disorientation, cramps of the arms, legs, and stomach, muscle fasciculation I. 2 were hospitalized II. 2 subjects were hospitalized for respiratory problems III. 4 were hospitalized Reported by HCP	I. RBC ChE: 32.5% Plasma ChE: 66.3% (p<.01 for both) II. RBC ChE: 5.6% Plasma ChE: 9.7% (p<.01 for all) (means) III. RBC ChE: 27.2% Plasma ChE: 29.4% (means) Michel Units	Study of 3 cases I. 23 fieldworkers went into a field 6h after 2 OPs applied. REI was 48 hrs. 16 became ill. 16 did follow-up up to 10 weeks. Baseline RBC ChE and Plasma ChE were determined when they stabilized in serial tests. II. 31 fieldworkers went into a field 2h after OP applied. REI was 48 hrs. 29 workers did follow-up for up to 12 weeks. Baseline RBC ChE and Plasma ChE were determined when they stabilized in serial tests. The Plasma ChE results of the day of exposure were not used since they were done in another lab and values were not comparable to the ones used in this study. ChE results used here were taken beginning one day after exposure. III. 18 workers on a mushroom farm were in a growing room when the entrance was sprayed with an OP. Symptoms developed after 15 minutes. 8 had RBC ChE and Plasma ChE initially done up to 48 hrs. later and follow-up tests 15 days later that were used as baselines. 4 others had Plasma ChE results on the day of exposure. They were not used since they were done in another lab and values were not comparable to the ones used in this study.

Study	Subjects*	Pesticides	Symptoms (and Signs)*	ChE Analyzed & % Inhibition from Baseline	Comments*
Subjects: Pesticide Plant Workers					
Kashyap, S. et al, (1984)	40 workers in formulation plant in India	Phorate	HA, giddiness, fatigue, N, V, stomach pain, skin and eye irritation, bradycardia	RBC ChE: -10% (elevated) Plasma ChE: 71% (means) RBC ChE: Post=104.39±19.40 Pre=95.00±13.21 Post/Pre=110% (p<0.05) Plasma ChE: Post=11.15±1.41 Pre=38.51±18.68 Post/Pre=29% (p<0.01)	Study of 40 workers in a formulating plant making 10% phorate granules from technical grade materials. Clinical exams, EKGs, and ChE were done before and after 2 weeks exposure. At 2 weeks, 60% had signs and symptoms. At 1 week, mean Plasma ChE 54% depressed but no symptoms recorded. ChE inhibition shown here was at 2 weeks.
			Reported by HCP	Voss and Sachsse Method	
Leng, G. et al, (1999)	262 chemical factory workers in Germany (127 in ethyl parathion accidents and 135 in propoxur accident)	Ethyl parathion Propoxur	Ethyl parathion: increased salivation, running nose, productive cough, visual disturbances, HA, GI irritation Propoxur: dizziness, lacrimation, sweating, tiredness, visual disturbances	Ethyl parathion accidents RBC ChE: 47-78% (Range of symptomatic) Propoxur accident RBC ChE: 29-64% (Range of symptomatic)	262 workers in a chemical factory were involved in handling accidents with ethyl parathion and propoxur. Ethyl parathion: 127 involved. Blood was drawn 30 minutes after exposures. 103 were symptomatic. Propoxur: 135 exposed after an accident. Blood was drawn 30 minutes after exposure. 100 individuals were symptomatic.

Study	Subjects*	Pesticides	Symptoms (and Signs)*	ChE Analyzed & % Inhibition from Baseline	Comments*
			Reported by HCP for propoxur subjects	Kinetic Enzyme/Substrate Measurements	
Mason, H., (2000)	8 symptomatic workers in a production plant making dichlorvos vaporization units in United Kingdom. There were 20 exposed workers.	Dichlorvos	"Flu like" symptoms, tiredness. Wheezing and chest tightness most prominent	RBC ChE: 57%-76% in the 8 symptomatic workers Plasma ChE: 85%-97% in the 8 symptomatic workers "Long established automated discrete kinetic method" (based on Ellman method)	20 workers in this plant were exposed when problems with production equipment developed and work shifts lengthened. 8 had symptoms. Measured dichlorvos in the air was 1.15 mg/m^3 (occupational exposure limit was 0.92 mg/m^3).
	*Abbreviations **Program**= California Medical Supervision Program **IPM**= integrated pest management		*Abbreviations **CP**=chest pain **GI**= gastrointestinal **HA**=headache **HCP**=health care professional **N**=nausea **SOB**=shortness of breath **V**=vomiting		*Abbreviations **REI**=restricted entry interval **NCV**= nerve conduction velocity **IPM**= integrated pest management

Studies of Agricultural Pesticide Applicators

In the five studies of workers who applied agricultural pesticides, one had the baselines established as part of the Program (Fillmore et al, 1993) and the other four studies' baselines were established during "low exposure" (Smit et al, 2003) or "low application" (Jintana et al, 2009) periods or "pre-spraying" seasons (Khan et al, 2010, Pathak et al, 2013). There were 508 subjects in these five studies. Three studies measured both RBC ChE and Plasma ChE (Fillmore et al, 1993, Jintana et al, 2009, Pathak et al, 2013), one study (Smit et al, 2003) measured only RBC ChE, and one study (Khan et al, 2007) measured only plasma ChE. The range of RBC ChE inhibition was 0-55% and the range of plasma ChE inhibition was 9-87%. See table D1d for the signs and symptoms reported for the subjects in these studies.

Studies of Pesticide Plant Workers

There were 322 subjects in the three studies of pesticide plant workers. Two studies measured both RBC ChE and plasma ChE (Kashyap et al, 1984, Mason, 2000) and one study (Leng et al, 1999) measured only RBC ChE. The range of RBC ChE inhibition was 0-78% and the range of plasma ChE inhibition was 71-97%. One showed a range of >30% for RBC ChE and plasma ChE inhibition of its 20 subjects but only provided detailed information for eight of its subjects with the most serious symptoms (respiratory) whose range of RBC ChE inhibition was 57-76% and range of plasma ChE was 85-97% (Mason, 2000). There was not enough information on the other 12 subjects who probably had lower levels of ChE inhibition. The study that measured only RBC ChE had 262 subjects of which 127 were plant workers in accidents with ethyl parathion of which 103 were symptomatic and had a range of inhibition of RBC ChE of 47-78%. In that study, another 135 were plant workers in an accident with propoxur of which 100 were symptomatic and had a range of inhibition RBC ChE of 29-64% (Leng et al, 1999). See table D1d for the signs and symptoms reported for the subjects in these studies.

Study of Agricultural Harvest Workers

Only one study involved agricultural harvest workers. This study was of three incidents that involved 63 subjects who were agricultural crop harvesters (Coye et al, 1987). RBC ChE and plasma ChE were measured in all the incidents. Two of the incidents involved fieldworker crews who went into fields that were recently sprayed with Toxicity Category I OP pesticides to pick crops before they were allowed to enter because the Restricted Entry Interval had not expired. The third incident involved 18 workers on a mushroom farm working in a growing room when the entrance was sprayed with a Toxicity Category II OP pesticide. None of these workers had pre-exposure baseline ChE measurements. They did have serial follow-up ChE tests done. The levels of their ChE tests after they recovered were used as their baselines to determine the amount of ChE inhibition they experienced. The range of RBC ChE inhibition was 5.6-32.5% and the range of plasma ChE inhibition was 9.7-66.3% for these workers. See table D1d for the signs and symptoms reported for the subjects in these studies.

Factors Influencing the Association between ChE Activity Levels and Health Effects

A certain number of factors influence the correlation between ChE activity levels and health effects making comparison between studies difficult. These include the following factors:

a) Exposure free period
b) Possible Co-exposures
c) Timing of post-exposure ChE tests
d) Method used to measure ChE
e) Data reported
f) Non-specific symptoms

Exposure Free Period

Because of high inter-individual variability (Brock et al., 1990; Brock et al., 1991), an accurate baseline for an individual prior to exposure in the field is needed to determine post-exposure ChE inhibition as accurately as possible. If a subject was exposed to a ChE-inhibiting pesticide during the pre-exposure period when baselines are established, then this exposure can lead to underestimation of the actual post-exposure ChE inhibition. The ChE-inhibiting pesticide exposure free periods were not consistent or well described in the eight studies. The subjects in Coye et al (1993) had follow-up serial testing to establish their baselines. In the five studies of workers who applied agricultural pesticides, one had the baselines done as part of the California Medical Supervision Program (Fillmore et al, 1993). Even this study did not specify how long an exposure free period to ChE-inhibiting pesticides the workers had before establishing their baselines. It only mentioned that one of the five affected workers' baselines was established during the spraying season and could have been exposed immediately prior to establishing his/her baseline. The other four studies of workers who applied pesticides (Jintana et al, 2009; Khan et al, 2010; Pathak et al, 2013; Smit et al, 2003) had their pre-exposure levels taken during periods of "low exposure" or "low application" or "pre-spraying" seasons. These studies offered no information on whether some or all of these subjects actually were or were not exposed during these periods.

Possible Co-exposures

In one of the studies of pesticide plant workers (Kashyap et al, 1984), the period free of exposures to the pesticide phorate could have been as short as a week before baselines were established. Other OP pesticides and organochlorines were produced in this plant and there was no information on possible exposure to these pesticides. Only information on phorate exposure was offered.

Another possible problem is that although the subjects in three studies were mainly exposed to ChE inhibitors, they might have been exposed to other types of pesticides which could have contributed to their reported symptoms. In one study, pyrethroids and organochlorines were also applied (Khan et al, 2010). In a second study, cypermethrin and sulfur were also applied (Pathak et al, 2013). In a third study, in addition to phorate, organochlorines were also produced in the plant (Kashyap et al, 1984). Although these other types of pesticides can cause some of the same symptoms, they would not have contributed to the ChE inhibition.

Timing of Post-exposure ChE Tests

A possible problem in the study of agricultural harvest workers is that the post-exposure ChE inhibition might have been underestimated. In the first case of the report, half of the workers had their initial post exposure ChE tests done within 24 hours of their exposure and the other half had their initial post exposure tests done 11 days after their exposure (Coye et al, 1987). If they all had their tests done within 24 hours of exposure, then the amount of ChE inhibition would likely to have been greater. In the second case, post exposure ChE values on the day of exposure were not included in the study because the tests were done in another lab and values were not comparable to the ones used in the study (Coye et al. 1986). ChE results in this study were taken beginning one day after the exposure. If ChE results of the day of exposure were able to be included, the amount of ChE inhibition would likely have been greater. In the third case, half of the workers had their initial post exposure ChE tests done within 24 hours of their exposure and the other half had their initial post exposure tests 11 days after their exposure (Coye et al, 1987). If they all had their tests within 24 hours of exposure, then the amount of ChE inhibition would likely to have been greater.

Method Used to Measure ChE

The nine studies did not use the same method to analyze ChE. Five used the Ellman method, the recommended method for the California Medical Supervision Program. One used a “long established automated discrete method kinetic” method based on the Ellman method, one used the Michel method, one used a “kinetic enzyme/substrate measurement” method, and one used a method of Voss and Sachsse. Results of the Michel method are probably comparable to results determined by the Ellman method. Two studies that compared the two methods concluded they were both accurate, but the Ellman method was slightly more accurate and had a lower coefficient of variance (Askar et al, 2011 and Khalil et al, 2017). The “long established automated discrete method kinetic” method is probably comparable to the Ellman method since it is based on it. The method of Voss and Sachsse is a modification of the Ellman method, but no studies could be found comparing it to the Ellman or Michel methods. The accuracy of the “kinetic enzyme/substrate measurement” method could not be evaluated further due to lack of information about it.

Data Reported

Some studies in this review provided a range of ChE inhibition of individual workers (Fillmore et al, 1993; Khan et al, 2010; Leng et al, 1999; Mason et al, 2000) and some provided the means of ChE inhibition with (Pathak et al, 2013; Smitet al, 2003; Kashyap et al, 1984) or without (Jintana et al, 2009; Coye et al. 1987) information on the standard deviation (SD).

Non-specific Symptoms

The way symptoms were reported can also contribute to imprecision as to what levels of ChE inhibition would cause them since the discerptions of symptoms were qualitative and no information was given about their severity. Many of the symptoms reported were non-specific such as nausea, vomiting, headache, and dizziness and might have other causes. Although ChE inhibition can affect several organ systems and produce many symptoms, it

might not have caused all the non-specific symptoms reported in these studies. Another factor that might influence outcome is the fact that symptoms were not always reported by a health care professional. In three studies, symptoms were self-reported (Jintana et al, 2009, Pathak et al, 2013, and Smit et al, 2003) and in one study (Mason et al, 2000) it was unclear if symptoms were evaluated by a health care professional.

CONCLUSION

Overall, the studies selected in this review demonstrated that exposure to OP/CB pesticides is associated to both a range of inhibition of RBC and plasma ChE and a wide range of symptoms and signs. The most common symptoms reported were dizziness, visual problems, headache, nausea, vomiting, and other GI symptoms, respiratory symptoms, and fatigue. Numerous other symptoms were also reported less frequently.

As highlighted in this review, interpretation of these study results to make any firm determinations as to which levels of ChE inhibition would cause symptoms is difficult because of differences in the following:

- the study design
- what was considered ChE-inhibiting pesticide exposure free periods
- when post-exposure ChE testing was done
- the method used to measure cholinesterase, ChE measured (RBC or plasma ChE)
- how data were reported (mean vs. range)
- the qualitative nature of how the symptoms were described

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2. Literature Review on Exposure to Organophosphate and Carbamate Pesticides and Cholinesterase Activity Levels

OBJECTIVE

The objective of this review is to identify pertinent studies in the peer-reviewed literature linking ChE activity depressions due to exposure to OP and/or CB pesticides to adverse health outcomes in order to validate the use of ChE levels as a biomarker of effect.

METHODS

PECO Statement

We created a Population, Exposures, Comparator and Outcome (PECO) statement, a modified version of Cochrane's PICO principle (Vandenberg, 2016), to guide our search.

Element	Explanation
Population (P)	Human subjects only, occupational setting only
Exposure (E)	Organophosphate and Carbamate pesticides, Toxicity Categories I and II
Comparator (C)	ChE activity levels during high exposure period versus ChE levels during low- and/or no-exposure period.
Outcome (O)	Depression of RBC or plasma ChE relative to individual baseline.

Search Strategy

The PubMed research database (https://www.ncbi.nlm.nih.gov/pubmed/) was used to identify relevant studies published any time up to May 2020 and was limited to full articles available in English. The search strategy involved using a combination of three domains. The first domain consisted of exposure terms, which are cholinesterase inhibiting pesticides. The second domain contained comparator terms affiliated to cholinesterase activity levels. Finally, in the third domain there were population terms such as occupational settings.

PubMed Search Terms

((pesticide*[tiab] OR pesticides [mh] OR insecticide*[tiab] OR herbicide*[tiab] OR Organophosphates [Mesh] OR "organophosphorus compounds"[mh] OR "organophosphate poisoning"[mh] OR Carbamates [Mesh] OR carbamate*[tiab] OR organophosphorus*[tiab] OR organophosphate*[tiab] OR chlorpyrifos [tiab] OR malathion [tiab] OR methomyl [tiab] OR triazophos [tiab] OR monocrotophos [tiab] OR profenofos [tiab] OR acephate [tiab] OR benzyliden [tiab] OR dichlorvos [tiab] OR diazinon [tiab] OR dimethoate [tiab] OR disulfoton [tiab] OR ethephon [tiab] OR ethoxyprop [tiab] OR fenamiphos [tiab] OR methyl parathion[tiab] OR naled [tiab] OR oxydemeton [tiab] methyl [tiab] OR phorate [tiab] OR phosmet[tiab] OR tributyl phosphorotrithioate [tiab] OR propetamphos[tiab] OR

tetrachlorvinphos [tiab] OR trichlorfon[tiab] OR carbamate*[tiab] OR carbaryl [tiab] OR carbofuran[tiab] OR chlorophen [tiab] OR cyclase [tiab] OR desmedipham [tiab] OR methiocarb [tiab] OR oxamyl [tiab] OR phenmedipham [tiab] OR propamocarb hydrochloride [tiab] OR sodium dimethyldithiocarbamate [tiab] OR thiobencarb [tiab] OR triallate [tiab]) **AND** (cholinesterase*[tiab] OR cholinesterases [mh] OR “cholinesterase inhibitors”[mh] OR acetylcholinesterase*[tiab] OR "ache"[tiab] OR Butyrylcholinesterase[tiab] OR Plasma ChE [tiab] OR "blood ChE"[tiab] OR “serum ChE”[tiab] OR Pseudocholinesterase* [tiab] OR “RBC ChE”[tiab] OR “Plasma ChE”[tiab]) **AND** (occupational exposure[mh] OR farmers[mh] OR occupational[tiab] OR job exposure*[tiab] OR worker*[tiab] OR farmer*[tiab] OR farmworker*[tiab] OR handler*[tiab] OR applicator*[tiab] OR sprayer*[tiab]) **AND** (eng[la] NOT (animals[mh] NOT humans[mh]))0

Study Selection

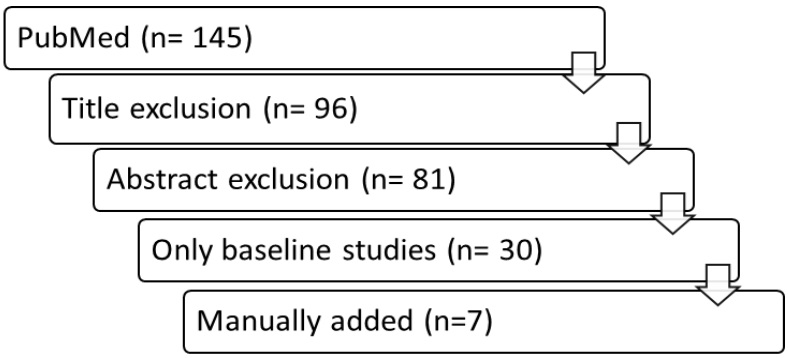
The PubMed search using the search terms described above resulted in 145 articles. The exclusion process aims to omit articles that are irrelevant to our PECO statement or those that do not provide the type of information we are looking for (Table D2a). Articles that were categorized as literature reviews, editorials, methodological articles and meta-analysis were excluded. Articles with no text available were also excluded (n=2). Only literature in English were examined. Studies with only adult human subjects, as well as those that evaluated OP and CB pesticide exposure and reported on either RBC or plasma ChE activity levels were selected for screening.

Table D2a: *List of exclusion criteria.*

Exclusion Criteria
1. Reviews, editorials, methodological articles, and meta-analyses
2. Not in English
3. Articles with no text
4. Animal studies
5. No OP and CB pesticide exposure
6. No RBC or plasma ChE
7. Not occupational

The initial phase of this process was to omit irrelevant articles by title and abstract analysis. The second phase was to omit articles based on their full content (Table D2b). The third phase was to only select articles for which baseline levels were available. Finally, frequently cited studies in the literature that were not in our literature pool were manually added (n=7).

Table D2b: *Exclusion criteria flow chart.*



Critical Appraisal of Studies

Each journal article of the 37 studies selected for review was assessed for its data quality using the following criteria: studies with a small sample size (n < 10), studies that did not specify if personal protective equipment were worn or not, and those that used a normal range or maximum ChE level as a baseline were removed.

Since the dose-response relationship between pesticide exposure and ChE depression is important to establish the level(s) of depression indicative of exposure, we focused on studies that had dose information or data on another metric of potential exposure (e.g., serum pesticide metabolite levels, foliage residue etc.).

Ultimately, only six studies of the 145 results of the PubMed search were found relevant and useful in our literature search.

RESULTS

All six studies looked at the effects of exposure to ChE-inhibiting pesticides on blood ChE levels of workers and had values for RBC ChE and/or plasma ChE for each subject (see Table D2c). A wide range of pesticide-induced ChE depressions was documented. ChE depression in exposed individuals ranged from marginal to severe.

Pesticide Exposure

Different indicators can be used to estimate exposure. Urine and serum metabolites are reflective of internal dose and are commonly used to estimate total exposure. Dermal exposure is a common route of pesticide exposure in workers, thus the pesticide concentration detected on workers' skin, is frequently used to examine the relationship between ChE depression and pesticide exposure. Air-sampling pumps are used to estimate exposure via inhalation, another common route of pesticide exposure.

Data related to exposure varied between studies. He et al. (2002) directly reported concentration of active ingredients as percent by volume of pesticide sprayed (0.23% for methamidophos and 0.35% for methylparathion). Wicker et al. (1979) measured residue levels on crops and found residue levels ranging from 0.13-0.35 µg/cm^2 (corn) and 0.03-2.36 µg/cm^2 (peaches). Others measured pesticide and/or its metabolite concentration in subjects' serum. Leng & Lewalter (1999) measured ethylparathion (530-650 µg/l plasma);

ehtylparaoxon (<10 - >100 µg/l plasma), and propoxur (490-960 µg/l plasma). Similarly, Muttray et al. (2006) measured methylparathion in serum (12.1 µg /l plasma). Drevenkar et al. (1991) looked at metabolites in the urine and found dialkyl phosphorus metabolites (DMTP, DMDTP, DEP. DETP, DEDTP) in urine ranging 0.02-8.27 nmol/mg creatinine on the first year 1 and 0.14-55.9 nmol/mg creatinine on the second year. Two studies (Karr, 1992; Muttray, 2006) measured levels in skin. Karr et al. (1992) reported total amount of pesticide detected on skin (19-1235 µg per person) using fluorescent brilliant sulfoflavin-soaked filter papers that were fixed on the subjects' skin. Muttray et al. (2006) reported total amount of pesticide detected on skin (2 ug-12 mg per person) using dermal pads with fluorescent tracers. Inhalation was measured in one study using air-sampling pumps worn by subjects and determined an average respiratory exposure of 22 µg per person (Muttray, 2006).

ChE Activity Level

In all six selected studies, depression was measured by comparing ChE activity levels during or just after exposure to individual baseline levels. However, the ChE depression information was reported in the literature in different ways. In two studies, ChE depression levels have been simplified to cut-off levels and were reported as below or above a certain threshold of clinical or health significance. In such cases, ChE activity measurements were not always reported. Of the two studies that met the exclusion and inclusion criteria, one study considered 20% and 30% for both RBC ChE and plasma ChE (Wicker, 1979) while the other study (Drevenkar, 1991) only evaluated plasma ChE depression and used cut-off thresholds of 10 and 30% depression. In Drevenkar et al. (1991), 42 individuals had plasma ChE depression of <10%, 26 between 11 and 30%, and 12 between 31 and 48%. In Wicker et al. (1979); 14 of the 33 sweet corn harvesters had plasma ChE depressions of >20% and 6 workers had depressions of > 30%. While 13 of the 33 sweet corn harvesters had RBC ChE depressions of >20% and 3 workers had depressions of >30%. No depression over 20% of either RBC ChE or plasma ChE was observed for peach blossom thinners.

Table D2c: *Summary of all six studies included in this review.*

Study	N	Location	Time period	Pesticide Exposure			ChE Analyzed & % Inhibition from Baseline				Characteristics of Individuals		
				Name	Dose	Duration	RBC ChE	Plasma ChE	Pre exposure blood collection	Post exposure blood collection	Demographics	PPE	Activities
Studies with threshold													
Drevenkar et al. (1991)	85 exposed and 12 controls	Yugoslavia	Spring & Summer of 1986 & 1987	methidathion & vamidothion (1986), azinphos-methyl (1987)	dialkyl phosphorus metabolites (DMTP, DMDTP, DEP. DETP, DEDTP) in urine: 0.02-8.27 (year 1) and 0.14-55.9 (year2) nmol/mg creatinine	2-4 days applications 2-4 weeks apart during spring and summer	NA	42 < 10% depression, 26 between 11 and 30%, and 12 between 31 and 48%.	six months after the last spraying	after the end of the third spraying session	adult males and females	mixers: gloves and masks); sprayers (cabin in tractor); field workers (gloves)	Orchard pesticide handlers (mixers, sprayers, and field workers)
Studies without threshold													
He et al. (2002)	90 exposed + 42 controls	Jiangsu and Shandong (China)	two years study	Methamidophos, methylparathion, and combined with Deltamethrin (pyrethroid)	concentration by volume of pesticide sprayed: 0.23% (methamidophos) -0.35% (methylparathion)	2-h exposure	21.30±7.82% for methamidophos and 14.59±7.04% for methyl parathion	NA	before spraying	just after spraying and 1 hour after	adult males and females	No	farmers spraying pesticides

Study	N	Location	Time period	Pesticide Exposure			ChE Analyzed & % Inhibition from Baseline				Characteristics of Individuals		
				Name	Dose	Duration	RBC ChE	Plasma ChE	Pre exposure blood collection	Post exposure blood collection	Demographics	PPE	Activities
Karr et al. (1992)	48 exposed (applicators) and 40 controls (slaughterhouse workers and supervisors)	Yakima Valley, Washington, US	Jan-Feb 1988 vs Aug-Oct 1988	azinphosmethyl, chlorpyrifos, phosphamidon	total estimated dermal exposure for 6 individuals only: 19-1235 ug per person	Two groups: <10days spraying and >10days spraying	mean = 3.9%	mean = 0.7%	at least three months after the last spraying	at least three weeks after the last application	unknown	Yes (1) rain suits with respiratory protection, 2) enclosed cabins and 3) gloves/hats/ respirators for exposure study only)	orchard applicators and mixers/ loaders
Studies without threshold													
Leng & Lewalter (1999)	workers exposed to ethylparathion (N-169) and propoxur (N=158) and controls (N=440 workers exposed to cyfluthrin)	Germany	NA	methylparathion, ethylparathio, propoxur and mixture with cyfluthrin	530-650 ethylparathion (ug/l plasma); <10 - >100 ehtylparaoxon (ug/l plasma); propoxur 490-960 (ug/l plasma)	2 incidents (no exposure duration available) and 2 non-incident studies ("regularly" for ethylparathion and 4 hrs for propoxur)	Group 1: 9-78%; Group 2: 2-49%; Group 3: not measurable; Group 4:17-64%	Group 1: 12-100%; Group 2: NA; Group 3: not measurable; Group 4:NA	during pre-employment medical examination	30 minutes after the accidents; not available for the non-incident studies	unknown	Yes	workers in chemical industry
Muttray et al. (2006)	23 sprayers	Germany	June-August	methyl parathion	Dermal: 2 ug-12 mg (max); inhalation: 22 ug (max); pesticide in plasma 12.1 ug /l (max)	50 min.	calculated from raw values: -12.95% ± 19.6	calculated from raw values: -5.21% ± 7.86	no OPs or neurotoxics used at least one week prior to baseline and weeks prior to spraying	43–82 min after spraying	adult males	Most wore PPE clothes, but no gloves and no closed cabins	wine growers sprayers

Study	N	Location	Time period	Pesticide Exposure			ChE Analyzed & % Inhibition from Baseline				Characteristics of Individuals		
				Name	Dose	Duration	RBC ChE	Plasma ChE	Pre exposure blood collection	Post exposure blood collection	Demographics	PPE	Activities
Studies with and without threshold													
Wicker et al. (1979)	sweet corn: 33; peaches: 30; Total: 63	North Carolina, US	April-May 1977 (peaches), 11 h/d; June - July 1977; 5-6 d/wk; 8 h/d (sweet corn)	parathion, methyl parathion, and paraoxon residues	0.13-0.35 ug/cm^2 (outer shuck); 0.03-2.36 ug/cm^2 (peaches); range incl. all pesticides; Urinary *p*-nitrophenol 0.1-0.7ppm	Corn: 21 applications done during harvesting season and field last treated 2 days prior to activity; Peach: 6 applications done on peaches during thinning season although 2 applications done prior to fruit thinning but weeks prior to activity	3.2-12.5 (peaches); 15-18 (sweet corn); mean: 8.6 -------- AChE: 13 of 33 workers had >20% depression and 3 > 30% (sweet corn); No depression over 20% (peaches)	0.4-21.5 (peaches); 13-23 (sweet corn); mean: 8.9 -------- 14 of 33 workers had >20% depression and 6 > 30% (sweet corn); No depression over 20% (peaches)	1 sample during blossom-thinning (peach); day before harvest started (corn)	3 samples during fruit-thinning (peach); 2 times two weeks apart during harvest (corn)	8-62 yrs old males and females	All wore PPE clothes, but only 40% wore cotton or rubber gloves	sweet corn packers (high and low contact groups) and peach thinners

Correlation between Pesticide Exposure and ChE Activity Level

Most studies found a significant correlation between exposure and ChE activity. Wicker et al. (1979) found a significant decrease of RBC and plasma ChE activity levels for corn packers but not peach thinners indicating that activity patterns play a role for dermal exposure of re-entry workers. Karr et al. (1992) found that RBC ChE was strongly correlated with dermal exposure measurements of applicators who sprayed pesticides for more than ten days even when using specific PPE. He et al. (2002) found that RBC ChE activity is highly correlated with the intensity and duration of higher exposure to OP/CB pesticides. Leng & Lewalter (1999) measured serum levels of ethylparaoxon, ethylparathion and propoxur and observed a correlation between serum levels and RBC ChE inhibition. However, two studies did not find a significant correlation between exposure and ChE activity. Muttray et al. (2006) did not see any significant depression of both RBC ChE and plasma ChE after exposure to methylparathion. Similarly, Drevenkar et al. (1991) did not observe a significant relationship between metabolite concentration in urine and plasma ChE activity levels.

Factors Influencing the Correlation between Pesticide Exposure and ChE Activity

A certain number of factors influence the correlation between pesticide exposure and ChE depression making comparison between studies difficult. These include the following factors:

a) Pesticides used
b) Study design
c) ChE methods
d) Characteristics of individuals

Pesticides Used

The different OP pesticides evaluated in the six studies could have varying effects on ChE activity levels. It is reasonable to believe the effect of these pesticides on blood ChE is similar to brain AChE. US EPA (2002) developed relative potency factors (RPF) for brain AChE inhibition as the measure of potency. For each route of exposure, the range of RPFs is very wide as some pesticides are much more potent than others. For example, for the oral route, US EPA found that for the same level and duration of exposure the relative toxicity of Aldicarb (4)>>Chlorpyrifos (0.06)>>Malathion (0.0003).

Study Design

Ideally, baseline should be taken when individuals have not been exposed to any ChE inhibitors for a certain period to assure that the levels measured are true baseline. However, the duration of exposure free period varies widely between studies from days to months. In Drevenkar (1991) pre-exposure samples were collected one month before the beginning of the spraying season, which was six months after the last spraying session. In Karr et al. (1992), baseline was taken at least three months after the last spraying. In Muttray (2006), baseline was taken after one week of exposure free period. In Wicker et al. (1979) for corn harvesters' baseline was taken a day before harvest started and for peach thinners baseline was taken during blossom-thinning operations prior to application. In Leng & Lewalter (1999), pre-exposure blood collection was taken during pre-employment medical examination, but no exposure free period was specified.

Similarly, the duration of exposure greatly affects the level of inhibition of the enzymes and varies widely between studies. While in Muttray et al. (2006) and He at al. (2002) subjects were exposed to ChE inhibitors to very short periods (50min. and 2h, respectively), while in the other four studies, individuals were exposed for multiple days, weeks or even a full season.

Finally, the interval between exposure to pesticides and blood collection greatly affects test results and varies widely between studies. Plasma ChE is expected to be inhibited quickly and recover quickly whereas RBC ChE is slow to inhibit and slow to recover. Therefore, study design will determine at which stage of inhibition and recovery the two enzymes are at the time of blood collection. Considering this factor is essential to accurately estimate exposure. In the six studies, the blood was drawn at different times after exposure. For example, in Karr et al. (1992) the blood was collected at least three weeks after the last application whereas in He et al. (2002) and Muttray et al. (2006) it was collected less than 2 hours after spraying.

Cholinesterase Methods

As mentioned earlier, the patterns of activity and recovery vary between plasma ChE and AChE. Plasma ChE is quick acting and recovers in 2-3 weeks while RBC ChE is slow acting and slow at recovering (2-3 months). In addition, some pesticides have more affinity for one of the enzymes than for the other. One study only looked at plasma ChE (Drevenkar, 1991), one study focused on RBC ChE (He, 2002), while the other four studies looked at both enzymes (Karr, 1992; Leng, 1999; Muttray, 2006; Wicker, 1979).

Different methods can be used to measure ChE activity levels in blood. In this review, we only selected studies that used the Ellman method, which is the same method used in the Program. However, only four studies clearly stated using the Ellman method to analyze ChE levels. Karr et al. (1992) used a field colorimetric method “based on Ellman” and He et al. (2002) used a field method based on the Ellman method.

The treatment of samples during collection and transport is another criterion that can affect test results. Some studies clearly described this step in the methods (Wicker, 1979; Karr, 1992; Muttray, 2006; Drevenkar, 1991) while others did not give sufficient details to be compared to the other studies (Leng, 1999; He, 2002).

Characteristics of Individuals

Inhibition of ChE activity levels by OP/CB depend on the specific activities performed by individuals. Some activities such as corn harvester and mixer/loader result in exposure by direct contact of the skin. Whereas working indoor or spraying pesticides on the field result in exposure by breathing pesticides present in the air. Four of the six studies are of pesticide handlers in agricultural fields and therefore workers are exposed during handling. In Wicker et al. (1979), peach thinners and corn harvesters entered the fields after application and were therefore not present during spraying. For these workers, only dermal exposure is expected. In Leng and Lewalter (1999), individuals work in chemical industry, so they are exposed indoor and at higher amounts.

The use of PPE significantly affects the level at which individuals are exposed. In one study (He, 2002) workers did not wear any PPE. In the other five studies, the level of protection varied widely. Some workers had minimal protection including gloves and long sleeves (Murray, 2006; Wicker, 1979) while others wore full gear including gloves, masks and even closed cabin (Drevenkar, 1991; Karr, 1992). Leng & Lewalter (1999) does not specify which PPE were used.

Age is an important factor influencing ChE activity levels, as ChE activity tend to decrease over lifetime (Brock, 1990). Although we focused our review on adults only, Wicker et al. (1979) had a wider range of individuals included in the study (8-62). Karr et al. (1992) and Lend & Lewalter (1999) did not specify the age of their study population. We assumed all individuals in these two studies from US and Germany were adults since they handled pesticides where children are not allowed to work with highly toxic pesticides. Gender is also an important factor influencing ChE activity levels as ChE activity in males is on average higher than in females (Brock, 1990). Wicker et al. (1979), He at al. (2002) and Drevenkar et al. (1991) included both males and females. Muttray et al. (2006) only included males. The other two studies (Leng, 1999; Karr, 1992) did not specify the gender of individuals who participated. Although gender and age may affect ChE levels of individuals, age- and sex-related differences in sensitivity to OP/CB pesticides should be minimal when comparing ChE levels to individual baseline levels.

CONCLUSION

All six studies selected in this review concluded that exposure to OP/CB pesticides induces a decrease in plasma and RBC ChE activity levels. This effect depends on many factors such as the relative toxicity of the chemical and the affinity of the pesticide for a specific ChE, the route, the amount and the duration of exposure, the characteristics of the individuals exposed and their activity patterns. Although this literature review only identified six studies because they matched all the inclusion criteria, there is a wider pool of literature on the topic that also converge to the same conclusions. However, many of the existing studies do not use individual baselines but rather control group of unexposed individuals and most studies do not provide any quantitative information on exposure levels.

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3. Literature Review of Intra- and Inter-individual Variations of Cholinesterase Activity Levels in Healthy Adults with no Exposure to Cholinesterase Inhibitors

OBJECTIVE

The objective of this short review is to analyze the existing peer-reviewed literature on intra- and inter-individual variations of both RBC and plasma ChE activity levels in the population of working adults with no known exposures to ChE-inhibiting chemicals for the purpose of validating the requisite for regular baseline levels to which follow-up must be compared to. This review also explores the physiological evidence for such variations.

METHODS

The PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) database was searched to identify relevant studies on pesticide exposure. A Population, Exposures, Comparator and Outcome (PECO) statement served as the guiding principal for the review process and aided in the classification of inclusion and exclusion criteria.

Element	Explanation
Population (P)	Human subjects (males and females), Healthy adult (18-65 yrs. old) only
Exposure (E)	No occupational exposure to any cholinesterase inhibitor prior and during study
Comparator (C)	Individual's levels over time (intra-individual) Variations between individuals of the same population (inter-individual)
Outcome (O)	Red blood cell or plasma cholinesterase activity levels measured using the Ellman method

Reviews, methodological articles and meta-analysis articles were excluded, and the literature was examined using exclusion criteria and critical appraisal. Ultimately, of all the results obtained from the PubMed searches, only six studies were found relevant for intra-individual variation and seven studies for inter-individual variations and used in our literature review.

RESULTS

Inter-individual Variation

Table D3a includes seven studies that analyzed inter-individual variation among ChE. The study populations included healthy laboratory staff, volunteers or other subjects without occupational exposure to known ChE inhibitors. These studies could be subdivided further based on the number of participants. Studies with fewer than 50 subjects may have larger coefficients of variation (CV) depending on the genetic homogeneity of the study population. For the larger sample size portion, the mean CV for RBC ChE was 13.91, ranging from 9.36 - 18.47. For plasma ChE, the mean CV was 23.45, ranging from 17.70 - 29.89. When combing both large and small sample size groups, the mean CV for RBC ChE was 7.47,

ranging from 0.36 -18.47 and the mean CV for plasma ChE was 19.12, ranging from 3.90 - 33.72. Not surprisingly, the inter-individual variation for ChE monitoring is quite large. Additional information for these studies can be found in table D3a. All studies demonstrate substantial inter-individual variations that were statistically related to physiological factors such as body weight, height, and gender, but are also influenced by a variety of other physiological and pathological conditions (Brock, 1990) and genetic variants (Lockridge, 2015).

Table D3a: *Literature review of inter-individual variation of cholinesterase activity level among non-exposed individuals.*

Study	Location	Method	Study Period	Study Subject Demographics	Gender	Subjects (M/F)	ChE	Coefficient of Variation
Karasova et al., (2017)	Hradec Kralove, Czech Republic	Ellman	Not stated	Healthy middle European (18 and 45 years)	M	201	RBC ChE	18.47
						193	Plasma ChE	23.57
					F	186	RBC ChE	15.54
						174	Plasma ChE	26.89
Worek (2016)	Munich, Germany	Ellman (mobile kit)	One time	Normal blood donors (18-61 years)	M	181	RBC ChE	9.36
							Plasma ChE	23.99
			One time	Normal blood donors (18-59 years)	F	61	RBC ChE	12.29
							Plasma ChE	26.15
Brock & Brock (1990)	University of Aarhus, Randers, Denmark	Ellman (and immunoassay)	First sample	Healthy volunteers with no occupational exposure to known inhibitors (19-65 years)	M	122	Plasma ChE	21.67
					F	71		20.37
Brock (1990)	University of Aarhus, Randers, Denmark	Ellman (and immunoassay)	Average of 6 samples over 8 months	Healthy volunteers without occupational exposure to known inhibitors (19-65 years)	M	40	Plasma ChE	20.85
					F	49		17.70
Moses (1986)	London, Ontario, Canada	Ellman	Once a week for six weeks and twice monthly for 10 months	Healthy laboratory staff (23-50 years)	F	24 (11/13)	Plasma ChE	29.89

Study	Location	Method	Study Period	Study Subject Demographics	Gender	Subjects (M/F)	ChE	Coefficient of Variation
Sidell et al., (1975)	Maryland, USA	Ellman	12 months	Healthy laboratory staff (23-67 years)	M	14	Plasma ChE	6.4
							RBC ChE	2.1
					F	8	Plasma ChE	6.1
							RBC ChE	3.1
			3 weeks	Healthy male soldiers (19-24 years)	M	9	Plasma ChE	3.9
							RBC ChE	1.5
Mason et al., (1989)	London, UK	Ellman	13 months Average of 6-11 samples per person	Healthy laboratory staff	M	7	Plasma ChE	10.03
							RBC ChE	6.06
					F	2	Plasma ChE	33.72
							RBC ChE	0.36
					M/F	9	Plasma ChE	15.60
							RBC ChE	5.95

** Green: studies of big sample size, Yellow: studies of small sample size,*

Studies	ChE	Mean	Median	Max	Min
Big sample size	RBC ChE	13.91	13.91	18.47	9.36
	Plasma ChE	23.45	23.57	29.89	17.70
Small sample size	RBC ChE	3.18	2.60	6.06	0.36
	Plasma ChE	12.63	8.22	33.72	3.90
ALL	RBC ChE	7.47	6.01	18.47	0.36
	Plasma ChE	19.12	20.85	33.72	3.90

** Green: studies of big sample size, Yellow: studies of small sample size, and Blue: all studies*

Intra-individual Variation

Table D3b includes six studies that analyzed intra-individual variation among ChE activity levels. These studies generally used healthy laboratory staff without occupational exposure to known ChE inhibitors or other normal-weight subjects. Among these six studies the observed intra-individual variation mean for plasma ChE was 5.3% with a range of 3.9 - 6.4%. For RBC ChE, the mean observed intra-individual variation was 3.18% with a range of 1.5 - 6%. The relatively low observed intra-individual variation for ChE between subjects and studies shows that variability is less of an issue for the California Medical Supervision Program due to the required practice of establishing individual baseline. Additional information for these studies is shown in Table D3b.

Table D3b: *Literature review of intra-individual variation of cholinesterase activity level.*

Author	Location	Method	Study Period	Study Subject Demographics	Gender	Subjects (M/F)	ChE	Observed intra-individual variation (%)
Brock (1990)	U of Aarhus, Randers, Denmark	Ellman, immunoassay	6 samples over 8 months	Healthy individuals without occupational exposure to known inhibitors (20-65 years)	-	94(43/51)	Plasma ChE	6.4
Brock & Brock (1990)	U of Aarhus, Randers, Denmark	Ellman, immunoassay	6 samples over 8 months	Healthy volunteers without occupational exposure to known inhibitors (19-65 years)	-	193 (122/71)	Plasma ChE	5.0
Moses (1986)	London, Ontario, Canada	Ellman	Once a week, for 6 weeks, twice monthly for 10 months	Healthy laboratory staff (23-50 years)	-	24 (11/13)	Plasma ChE	4.2
Mason et al., (1989)	London, UK	Ellman	13 months	Healthy laboratory staff	-	9 (7/2)	Plasma ChE	4.7
							RBC ChE	6.0
Hȍlzel, (1987)	Berlin, Germany	Ellman	Once a week, for 8 weeks	Normal-weight (men: 31-50 years) (women: 20-44 years)	M	10	Plasma ChE	5.7
					F	14		4.6
Sidell et al., (1975)	Maryland	Ellman	Twice monthly, for 1 year	Healthy laboratory staff (23-67 years)	M	14	Plasma ChE	6.1
							RBC ChE	2.1
				Healthy laboratory staff (23-67 years)	F	8	Plasma ChE	6.4
							RBC ChE	3.1
			5 days/week, for 3 weeks	Healthy male soldiers (19-24 years)	M	9	Plasma ChE	3.9
							RBC ChE	1.5

ChE	Mean	Median	Max	Min
Plasma ChE	5.3	5.35	6.4	3.9
RBC ChE	3.175	2.6	6	1.5

Depression of ChE activity can be significant in that it is outside the degree of variation seen in normal individuals or in that relates to the onset of adverse health effects (Mason et al., 1989). A decrease in an individual's enzyme activity signifies excessive exposure if it is clearly greater than the normal intra-individual variation found in unexposed subjects (Mason et al., 1989). Researchers in the UK found that percentage drops between successive measurements that are greater than 15% and 7.5% for the plasma ChE and RBC ChE, respectively, suggest significant organophosphorus exposure to pesticide workers (Mason et al., 1989). Another study performed on a healthy population with no occupational exposure to known ChE inhibitors found that the intra- individual variations during an 8-month period varied substantially from one individual to another (3%-41% of the subject's mean activity) (Brock et al., 1990). Six consecutive ChE measurements in 95 individuals showed the distribution of maximum intra-individual variations of cholinesterase substance concentrations had a mean of 21% (range 6%-43%) (Brock, 1990). In a study that compared employees of an organophosphorus insecticide factory and a reference group, substantial intra-individual variation was shown to be up to 40% (Brock, 1991).

Physiological Evidence

Many studies exist on genetic differences and biological-based effectors have been reported to affect the expression, activity or degradation of these biomarkers. In the next two sections, we describe some of these differences.

Inter-individual Variation

Two separate genes regulate the tissue-specific expression of each ChE. While RBC-bound RBC ChE does differ structurally from the neuronal form of RBC ChE, both forms share the identical catalytic site as the coding DNA sequence for this region is highly conserved (Lockridge, 2016, Li, 1993). In a database of over 60,000 unrelated individuals, 278 mutations were detected within the coding exons of the human RBC ChE gene, yet only 3 loss-of-function mutations were found and all 3 individuals carried a single copy of the mutation (Lockridge, 2016).

In contrast, the same database of unrelated individuals revealed 293 plasma ChE mutations, 34 loss-of-function mutations and that 1 out of every 5 individuals carry the most common loss-of-function K variant (Lockridge, 2016). Individuals who are homozygous for the K variant have 33% lower plasma levels of plasma ChE protein. Less common mutations can result in succinylcholine sensitivity (carbamate-insensitive atypical variant), fluoride sensitivity or completely inactive forms of plasma ChE (Lockridge, 2016; Kalow, 1957). Reduced plasma ChE expression or activity results in a larger toxicant dose reaching the neuromuscular junction (Lockridge, 2016). Conversely, in rare cases, otherwise normal individuals had plasma ChE activity that was 2 to 3-fold higher than the general population (Neitlich, 1966).

Other serum proteins may indirectly affect ChE activity in blood. The serum esterase paraoxonase 1 (PON-1) hydrolyzes OPs and can reduce binding and inactivation of ChE (Aldridge, 1953, Costa et al, 2005). Serum PON-1 activity may vary as much as 40-fold within the population due to factors such as genetic variants, age, disease, diet or exposure to medications, alcohol, smoking (Mueller et al, 1983; Costa et al, 2005). Consequently,

individuals with low levels of PON-1 activity may be more sensitive to OP pesticides (Costa et al, 2005; Hofmann et al, 2009, Huen et al, 2010).

Intra-individual Variation

Due to genetic variation and post-translational effects, there is substantial intra- and inter-individual variation in RBC ChE and plasma ChE activity within the general population (Brock, 1991; Mason, 2000) such that a reference ChE baseline measurement for each individual is often necessary to accurately estimate the degree of ChE inhibition (Coye, 1987).

CONCLUSION

Due to genetic variation and post-translational effects, there is substantial inter-individual variation and modest intra-individual variation in RBC ChE and plasma ChE activity within the general population (Brock, 1991; Mason, 2000) such that a reference ChE baseline measurement verified regularly for each individual is necessary to accurately estimate the degree of ChE inhibition (Coye, 1987).

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Appendix E: Laboratories Approved for Cholinesterase Testing for Occupational Health Surveillance, April 16, 2019

State of California—Health and Human Services Agency
California Department of Public Health
Environmental Health Laboratory
Jed Waldman, PhD, Chief

KAREN L. SMITH, MD, MPH
Director and State Public Health Officer

GAVIN NEWSOM
Governor

Laboratories Approved for Cholinesterase Testing
for Occupational Health Surveillance, per California Code of Regulations*

This List is dated **April 16, 2019**;
it replaces and supersedes the List dated March 20, 2018.

ARUP Laboratories
500 Chipeta Way
Salt Lake City, UT 84108
(800) 522-2787
http://www.aruplab.com
CLIA No. 46D0523979
CA Lab No. COS 800007

Laboratory Corporation of America (LabCorp)
1447 York Court
Burlington, NC 27215
(336) 584-5171
https://www.labcorp.com
CLIA No. 34D0655059
CA Lab No. COS 00800058

Pacific Toxicology Laboratories
9458 De Soto Avenue
Chatsworth, CA 91311
(800) 328-6942
http://www.pactox.com
CLIA No. 05D0542735
CA Lab No. CLF 4442

Quest Diagnostics
3714 Northgate Blvd.
Sacramento, CA 95834
(866) 697-8378
http://www.questdiagnostics.com
CLIA No. 05D0644209
CA Lab No. CLF253

Quest Diagnostics – Nichols Institute
33608 Ortega Highway
San Juan Capistrano, CA 92690
(949) 728-4000
http://www.questdiagnostics.com
CLIA No. 05D0643352
CA Lab No. CLF 00002562

This list is *subject to change*; the current list is posted on-line at
https://www.cdph.ca.gov/Programs/CCDPHP/DEODC/EHLB/Pages/CDPH-Approved-Cholinesterase-Laboratories.aspx

Direct inquiries to Ms. Lissah Johnson,
Environmental Health Laboratory
850 Marina Bay Parkway, G365
Richmond, CA 94804
Lissah.Johnson@cdph.ca.gov

*CCR Title 3, Section 6728(f); see http://www.cdpr.ca.gov/docs/legbills/calcode/030302.htm

850 Marina Bay Parkway, G-365, Richmond, CA 94804
(510) 620-2803
http://www.cdph.ca.gov/programs/EHLB/

Appendix F: Laboratory and ChE Data Issues, Actions Taken, and Status

ISSUE	DPR ACTION	LABORATORY ACTION	STATUS
• All ChE test results throughout the state are reported to DPR, including ChE test results for medical, surgical and drug monitoring purposes.	• Continuous monitoring	• All laboratories informed DPR that it is difficult to pull out ChE test results under the medical supervision program because these data have no unique identifiers; there is no repository in the Laboratory Information System (LIS) for data required under the law.	• Unresolved. DPR continues to proactively work with the laboratories to improve data quality.
• Although the requisition slips have been modified, the laboratories continue to receive ChE test orders without indication (or ambiguous terms entered) of the purpose of test.	• Continuous monitoring	• Laboratories informed DPR that physicians could request them to modify the test order slips to include options for ChE tests and purpose of test. • 4 of the 6 laboratories added a checkbox (with ChE tests option) on the requisition slips. • Only 1 of the 4 laboratories was able to also include a checkbox of terminology for the purpose of test.	• 5 laboratories' requisition slips do not have a place to indicate purpose of test; 1 laboratory's requisition slips have been preprinted with purpose of test: Baseline, Periodic Monitoring, Post Exposure. • 2 laboratories continue to report purpose as unavailable.
• LIS is not programmed for transmission of HSC § 105206-required data; purpose of test is not transmitted from the laboratory that draws blood, to the laboratory that analyzes the blood specimen and ultimately reports the ChE test results to DPR.	• Continuous monitoring	• In 2013 and 2015, 2 of the 6 laboratories modified their online ordering system to allow physicians to enter (type) the purpose of test when ordering ChE tests.	• Unresolved
• In 2014–2019, 1,159 varieties of ambiguous terms were reported as *purpose of test.*	• DPR communicates with the reporting laboratories to ascertain the purpose of test, and to verify information on the ChE report.	• Laboratories report purpose of test that providers indicate. Otherwise, the purpose is reported as not given or unavailable.	• Unresolved
• In 2014–2019, ChE test records did not indicate the tested individual's employer and the name of the ordering medical supervisor.	• DPR communicates with the reporting laboratories to include the employer's and the ordering medical supervisor's names and contact information.	• Laboratories reiterated that they cannot report information they do not receive.	• Unresolved

ISSUE	DPR ACTION	LABORATORY ACTION	STATUS
• Data Entry Errors: Typographical Errors, Excel Spreadsheet columns switching	• Staff identifies these data errors and communicates with the laboratory.	• Laboratories resubmit corrected ChE report.	• Data entry errors continue to be identified in ChE reports.
• Increase in 2015 and 2016 baseline records	• Staff discussed with the laboratories and requested they verify purpose of test reported	• PALI was unable to verify the accuracy of the disproportionate increase in baseline record entries.	• PALI: February 2015 baseline records increased over 100% from records in February 2014. • QDI-SAC: 2015 baseline records increased over 100% from the baseline records in 2014.
• Decrease in follow-up records reported	• Staff discussed with the laboratories and requested to verify purpose of test reported	• Laboratories verified that the information submitted to DPR is what they had received.	• PALI: 2015 follow-up records decreased 49%; 2016 follow-up records decreased 43% compared to the previous year. • QDI-SAC: In 2016 follow-up records decreased greater than 100% from 2015 follow-up records.
• Two laboratories reporting the same ChE test results	• Staff verified with the two laboratories the apparent change in the reporting process. Staff manually sorted each laboratory's ChE records, saved duplicate records as a separate file, and saved ChE files as distinct reports in each of these two laboratory's respective folder.	• In December 2015, PALI began forwarding their blood specimens for analysis to an out of state laboratory (ARUP). • ARUP reported ChE test results for PALI.	• ARUP analyzed blood specimens drawn by PALI, and reports ChE test results for both laboratories, from 2016–2017. • PALI also reported the same ChE test result records that were reported by ARUP, from 2016–2017. • PALI discontinued ChE testing in 2018.
• Increase in total ChE records reported by PALI in 2016	• Staff reviewed each laboratory's ChE reports and determined the source of the increase of PALI records in 2016.	• PALI informed DPR that they report ChE test results records that ARUP transmits back to them	• In 2011–2015, PALI reported the required two ChE test types; ARUP analyzes ChE as a panel with 5 ChE test types. When ARUP began reporting for PALI, all 5 ChE test types were included in the PALI ChE records reported to DPR. This increased PALI's record count in 2016 (See Figure 2).
• Decrease in ChE records by QDI-SJC and increase in ChE records by QDI-SAC from 2018 to 2019	• Staff reviewed the laboratories' ChE reports and contacted QDI-SJC.	• QDI-SJC informed DPR staff that a client was no longer ordering ChE tests with QDI-SJC and is now ordering with QDI-SAC, accounting for the decrease in QDI-SJC records and increase in QDI-SAC records in 2019.	• A client ordering ChE tests through QDI-SAC, instead of QDI-SJC.

Appendix G: Cholinesterase Data Analysis

BACKGROUND

AB 1963 (Statutes of 2010, Chapter 369), enacted in 2011, stipulated several changes in California's Medical Supervision Program ("Program"). The law requires certified laboratories that analyze cholinesterase (ChE) activity in blood samples of employees who regularly handle organophosphate (OP) and carbamate (CB) pesticides Toxicity I and II, to report specific information pertaining to the test result, the employee, his or her employer, his or her physician, and the laboratory to DPR. In 2015, OEHHA and DPR evaluated the effectiveness of the Program and the utility of laboratory-based reporting, and developed recommendations that were presented in a report to the Legislature. Because of the recommendations in the 2015 Report, AB 2892 (Statutes of 2016, Chapter 475) was passed by the Legislature in 2016 (effective January 2017) to add new reporting requirements to improve the quality and quantity of the data being submitted. In addition, since January 2017, physicians under the Program are required to register with OEHHA and to report ChE depressions indicative of exposure to the local health officer within 24 hours. Both OEHHA and DPR have since performed a number of outreach efforts to employers and physicians. In this report, OEHHA analyzed the ChE test results received between 2014 and 2019 in order to give an update on the Program evaluation and to assess the effectiveness of the various efforts that have been carried out.

METHODS

Similar to the 2011–2013 ChE data that was analyzed in the 2015 Report, a large proportion of the data received from reporting laboratories between 2014–2019 were inaccurately classified or labeled, making it difficult to decipher the purpose of test (i.e. baseline, follow-up, recovery) and identify individuals. For this reason, similar to the 2015 Report, extensive cleaning and excluding of data was required (see 2015 Report for more details). Generally, the data cleaning and excluding process included correcting data that contained typographical errors and deleting those that contained incorrect information. Due to poor data quality and the high level of uncertainty around the accuracy of the data, data analysis was limited to macro-level assessments of the Program such as analyzing temporal and spatial trends on a regional level instead of assessments on a granular level, such as determining which individuals were and were not in compliance with the Program requirements.

After data cleaning, exclusion, and analysis, OEHHA identified individuals with ChE test results that exceeded the action level thresholds outlined in the *Guidelines for Physicians Who Supervise Workers Exposed to Cholinesterase-inhibiting Pesticides* (*Guidelines for Physicians*) and 3 CCR § 6728. Medical supervisors of these individuals were contacted to verify these test results, which revealed some issues with the way the ChE data were processed. This prompted certain adjustments to the data cleaning and analysis methodology, such as changes to how individuals and baselines were identified. These changes are described in detail in this section.

Data Preparation for Analysis

Cleaning the Data

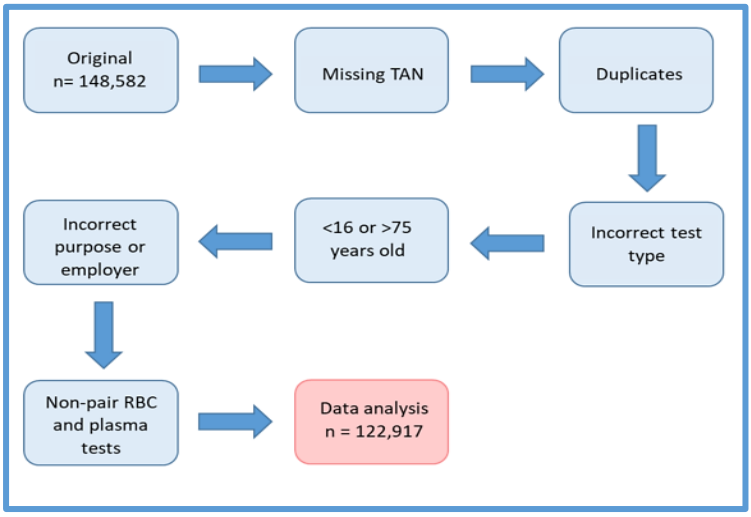


Figure G1: *Flow chart of exclusion process. Duplicates and missing information, such as test accession numbers (TAN), along with other information indicating ChE tests were not ordered under the Program were excluded.*

A large proportion of the data received by reporting laboratories contained errors. General cleaning of the data was conducted to fix and eliminate input errors, including typographical and duplicate tests. Tests with missing test accession numbers (TAN) were also removed from the dataset because it would not be possible to verify that the test was unique to an individual. Since this Program only collects red blood cell (RBC) and plasma ChE tests, tests that had incorrect test types (e.g. whole blood) were also excluded. This step remained similar to the 2015 Report (refer to 2015 Report, Appendix C, page 53 for more details). For this report, the R software and Microsoft Excel were used to clean the data.

Assigning Unique IDs

One major issue with the data quality was typographical errors in the names of individuals and physicians. This made it difficult to conduct data analysis because names in the raw data from the reporting laboratories could not be relied on for identifying unique individuals and medical supervisors under the Program. For example, it was often difficult to determine whether records with names of different spellings belonged to the same individual. For this analysis, we created a unique identifier for each individual and physician by measuring the similarities between names. For example, the R package stringdist[1] was used to measure the Jaro-Winkler distance between names. Names under a specific threshold of similarity (< 0.12) were assigned the same unique identifier and treated as a single individual. This method allows for identification of individuals without the need to change the original names, which is useful when investigating individual ChE depressions. This way OEHHA was able to compare IDs with individuals' names to ensure the correct individual was being analyzed.

Applying Exclusion Criteria

Exclusion criteria were developed and applied to the data to eliminate records that may be irrelevant to the Program (Figure G1). Similar to the criteria applied to the 2011–2013 data (see 2015 Report, Appendix C, page 54), individuals older than 75 and younger than 16 were likely not part of the Program, thus excluded. Tests that were not part of a pair of RBC

[1] van der Loo M (2014). "The stringdist package for approximate string matching." _The R Journal_, *6*, 111-122. <URL:https://CRAN.R-project.org/package=stringdist>.

and plasma ChE tests were also excluded. Furthermore, since ChE tests were also ordered for other purposes, tests containing information that indicated irrelevant purpose (e.g. “fire department”) were also excluded from the data.

DATA ANALYSIS

Baseline Estimation

After the data cleaning and exclusion process, baseline tests were identified for each individual. As previously mentioned, the majority of tests are labeled with an incorrect test purpose or missing this information. Thus, for those individuals , baseline estimates were determined as follow (Figure G2): For individuals with two tests 14 days apart during low pesticide use months, the average of these two tests was calculated and used as baseline (described below). For individuals who did not have 14-day baseline estimates, their maximum ChE values were used to extrapolate baseline estimates. For individuals who only had a single ChE test per year, baseline estimates and follow-up tests could not be determined. Thus, only spatial and temporal analysis was conducted for this pool of individuals.

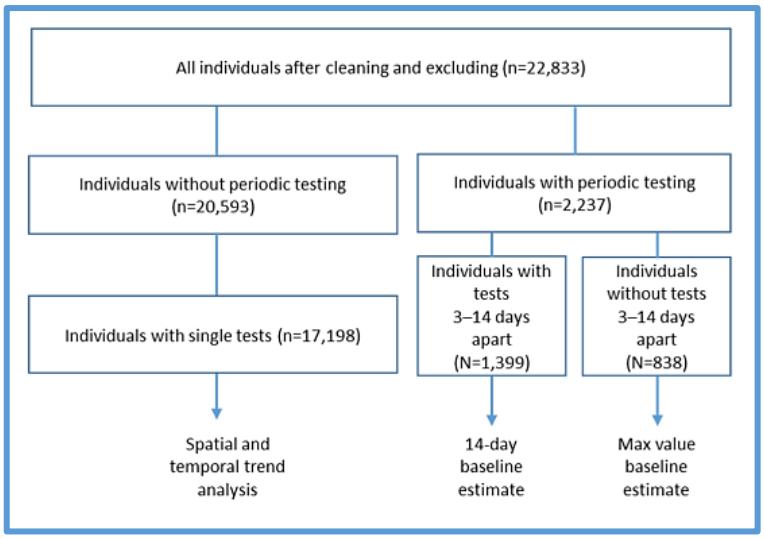


Figure G2: *Flow chart describing approaches to deriving baseline estimates for individuals with 14-day baseline estimates and those without (maximum ChE value).*

Determining Baselines for Handlers with 14-day Baseline Estimates

Similar to the 2015 Report, baseline tests were determined by identifying two tests taken within 3 to 14 days apart. Additionally, since the *Guidelines for Physicians* recommends baseline tests be taken within a 30-day exposure-free period, baseline determination was limited to tests ordered during low-pesticide-spraying months or a “low-spraying season.” In the 2015 Report, Pesticide Use Report (PUR) statewide data was used to identify a low-spraying season (November through March). However, spraying patterns on a smaller scale (e.g. region) may differ from state-level, thus the previous approach could have masked local low-spraying seasons that may more accurately reflect individual handlers’ work practices. In order to address this issue, region-level spraying patterns were analyzed using PUR data from 2014–2019.

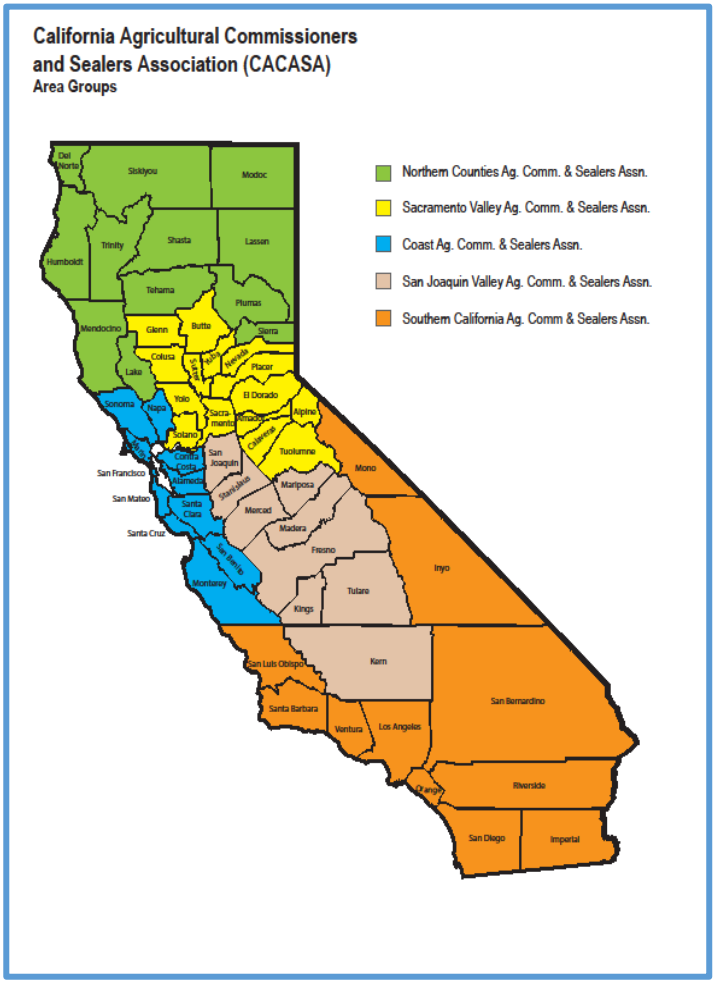


Figure G3: *Map of California Agricultural Commissioners and Sealers Association (CACASA) Area Groups.*

Determining Pesticide-use Patterns

The Program is limited to handlers who regularly handle pesticide products that contain an OP or CB as active ingredient and with the signal word "DANGER" (Toxicity Category I) or "WARNING" (Toxicity Category II). Therefore, the pesticide-use patterns for all OPs and CBs Toxicity I and II from 2014 through 2019 were analyzed on various levels (county, regional, and state). A correspondence with DPR staff familiar with local work practices revealed that handlers usually work in multiple counties within the same region, rarely moving between regions or across the state. Therefore, after initial county-level analysis of pesticide use, counties were grouped into geographic regions because county-level spraying patterns was determined to be too granular to use to be representative of handlers practices. To analyze pesticide spraying on a larger scale, the California Agricultural Commissioners and Sealers Association (CACASA) Area Groups were chosen because these five area groups are comprised of counties grouped by similarities in agricultural practices and issue areas: Northern Counties, Sacramento Valley, Coast, and Southern California (Figure G3).

Low-spraying months were determined for each area group. A low-spraying month was defined as a month for which pesticide usage is below half a standard deviation from the mean amount for the area group. Three consecutive low-spraying months or more constitute a low-spraying season. The statewide low-spraying season remained the same as what was reported in the 2015 Report. As shown in Figure G4, low-spraying seasons varied between area groups. The majority of tests (73.0%) with geographical information (i.e., zip codes) were from Coast and San Joaquin Valley area groups, therefore, for the purpose of this report, we focused our analysis to these two area groups.

The work location of pesticide handlers needed to be determined prior to using area group-level low-spraying seasons. Since 3 CCR § 6728 requires employers to send handlers under this Program to medical supervisors, it was assumed that handlers see medical supervisors local to where they work. Thus, physicians' zip codes were used to identify which region handlers worked in because this data element may be the optimal proxy measure of

handlers' work location. Employers' zip codes were determined not reliable for identifying the work location of handlers because this data field has often missing information or contains the zip code of a single office or corporate headquarters, which would not be an accurate determinant of where handlers were actually working.

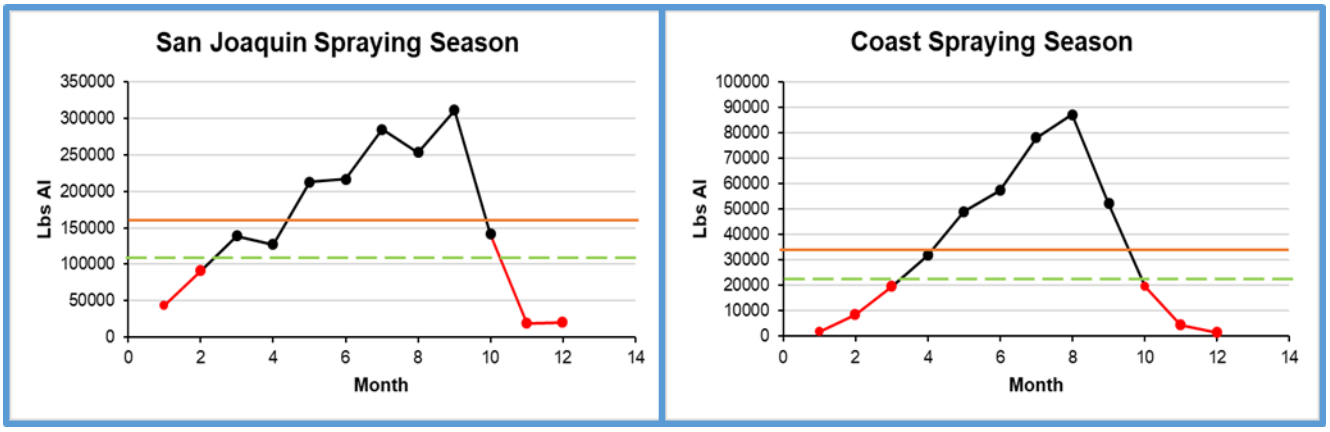


Figure G4: *Average monthly pesticide usage of all Type I and II OPs and CBs used from 2014 through 2019 (lbs. of active ingredient). The low-spraying seasons for San Joaquin (left) and Coast (right) area groups are represented in red. When the amount sprayed per month falls below half a standard deviation (green dashed line) from the mean (orange solid line), it was considered a low-spraying month. Three or more consecutive low-spraying months were considered part of a low-spraying season.*

Calculating Baseline Estimates

After the area groups for tests containing geographic information (i.e. physicians' zip code) were determined, two tests taken 3 to 14 days apart during the low-spraying season were selected as potential baseline tests. Initially, a baseline estimate was calculated for each patient by averaging baseline tests for each test type within a two-year period because, under 3 CCR § 6728, baseline estimates must be verified every two years by medical supervisors. However, OEHHA performed follow-ups of ChE depressions determined using the 2015 Report approach that revealed issues with this approach. Indeed, baseline estimates applied over a two-year period to calculate ChE depressions led to errors in ChE depression determination. One instance is that when a single ChE measurement taken in a year without 14-day baseline tests was treated as a follow-up test, but was actually a baseline test. That is because, even if baseline tests are required to be verified every two years, some physicians prefer to verify it yearly by taking a single test in the second year.

Thus, in the current analysis, 14-day baseline estimates were used to calculate ChE depression of the same year (i.e. a single spraying season). This approach may more accurately identify individuals' baseline tests that follow the *Guidelines for Physicians* (two tests 3–14 days apart within 30-day exposure free period), but fail to capture follow-up tests in the second year after baseline and therefore underestimate total number of ChE depressions.

Determining Baselines for Handlers without 14-day Baseline Estimates

Approximately half of the individuals with more than three tests per year do not have 14-day baseline estimates. Without baseline estimates, ChE depression cannot be determined. Although the *Guidelines for Physicians* recommends using an average of two tests taken 3 to 14 days apart to determine individuals' baseline, under 3 CCR § 6728 medical supervisors may only order a single test for each test type. Since the purpose of tests are often missing or incorrectly entered, it is difficult to identify which single ChE records are baseline tests. Three different approaches were applied in order to identify possible proxy baseline estimates.

Removing Outliers

Similar to the approach used in the 2015 Report, the three approaches all use maximum ChE values of each patient as a proxy measurement for baseline. However, the approach used in 2015 solely used individuals' maximum ChE values as baseline estimates, which may have led to an overestimation of ChE depressions. Thus, for this analysis, outliers were first removed using R.

Supplemental Study I:
Investigation of Different Ways to Establish Baseline using the Maximum ChE Values

Three approaches were investigated to estimate baseline for individuals **without identifiable 14-day baseline estimates**. Three approaches were investigated using the i**ndividuals with 14-day baseline estimates** and compared to the 3–14 day baseline estimates and the approach closest to the reference method was selected.

Approach #1: Maximum ChE Values

The first approach uses each individual's maximum ChE value as a baseline to calculate ChE depression levels (Figure G5). The mean difference between the maximum and baseline ChE values was 4%.

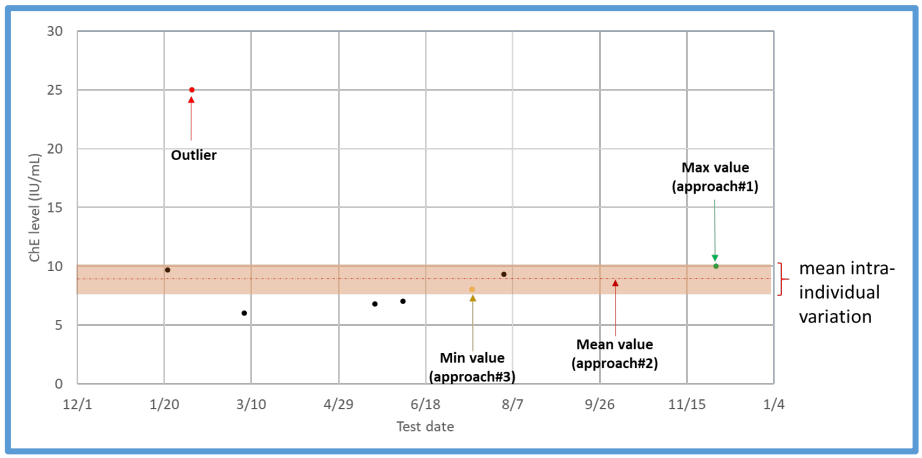


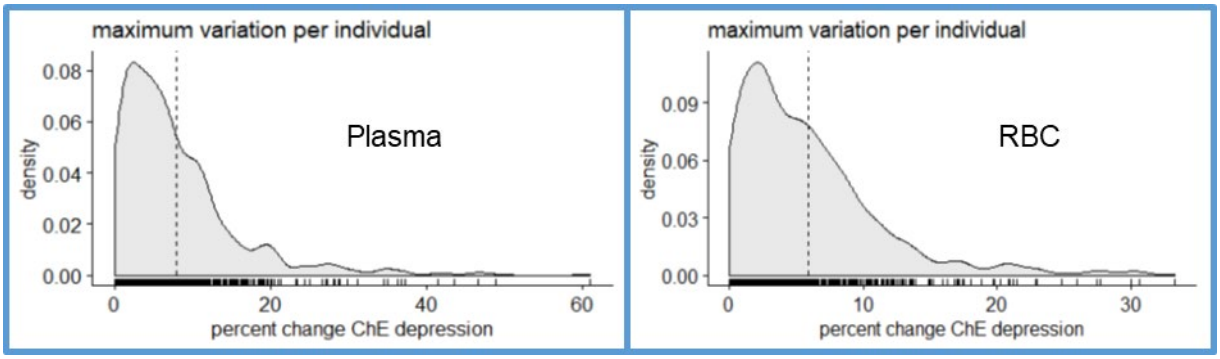
Figure G5: *An example of removing outliers and applying the three approaches using maximum value.*

Approach #2: Average within Intra-individual ChE Level Variability Range

The second approach calculates the variation between baseline tests of individuals with 14-day baseline tests (i.e., intra-individual ChE variation) and applies it to the maximum ChE values individuals without 14-day baseline tests to use as a proxy baseline estimates. A number of factors that are unrelated to pesticide exposure (e.g., diet, medication, etc.) can influence individual ChE activity levels.

In order to determine the intra-individual variation unrelated to pesticide exposure, the variation of individuals' baseline tests was calculated using data from individuals with 14-day baseline tests. The total variation of individuals' baseline tests were calculated as follows:

Percent intra-individual variation =



For both RBC and plasma, the mean intra-individual variation for this group of individuals was 8%. This value was used to estimate the average within intra-individual ChE level variability range for the group of individuals without identifiable 14-day baselines. For each individual, the mean of ChE values within this range was used as a baseline estimate.

Approach #3: Minimum within Intra-individual Variability Range

The third approach uses the minimum ChE test within the maximum ChE value and intra-individual variation range for each individual as the baseline estimate.

Comparing the Baseline Estimates using the 3 Approaches with 14-day Baseline Estimates

Of the three possible ChE values (i.e., maximum, average, and minimum) within the intra-individual variation range, the maximum value was chosen to extrapolate baseline estimates for individuals without identified 14-day baseline tests because the mean of max values was closest to the mean of 14-day baseline estimates.

ChE Depression Calculation

The same approach was used to calculate ChE depressions as described in the 2015 Report.

Spatial and Temporal Correlation Analysis

As done previously in the 2015 Report, spatial and temporal analysis was conducted using ArcMap GIS (geographic information system) software and Microsoft Excel. The association between the number of ChE tests ordered and pesticide usage per county, area group, and for the state was evaluated and the geospatial and temporal correlation was determined using Pearson's correlation test. The data used to assess the association was extracted from the PUR from 2014 through 2019. Only pesticide products with the signal word "DANGER" (Toxicity Category I) or "WARNING" (Toxicity Category II) that contain an OP or CB were used in this analysis (Table G1). Figure G6 shows a significant decline in pesticides usage over time.

***Table G1**. List of 35 Toxicity Categories I and II ChE-inhibiting pesticides used for agricultural purposes in California in amounts of 10 pounds or more between 2014 and 2019.*

Type I and II OP/CB Active Ingredients				
ACEPHATE	CYCLOATE	ETHOPROP	METHLY PARATHION	PHENMEDIPHAM
ALDICARB	DDVP	FENAMIPHOS	MEVINPHOS	PHORATE
BENSULIDE	DIAZINON	FORMETANATE-HYDROCHLORIDE	MEVINPHOS, OTHER RELATED	PHOSMET
BUTYLATE	DIMETHOATE	MALATHION	NALED	PROPETAMPHOS
CARBARYL	DISULFOTON	METHIDATHION	OXAMYL	S,S,S-TRIBUTYLE, PHOSPHOROTRITHIOATE
CHLORPROPHAM	EPTC	METHIOCARB	OXYDEMETON-METHYL	THIOBENCARB
CHLORPYRIFOS	ETHEPHON	METHOMYL	PARATHION	TRIALLATE

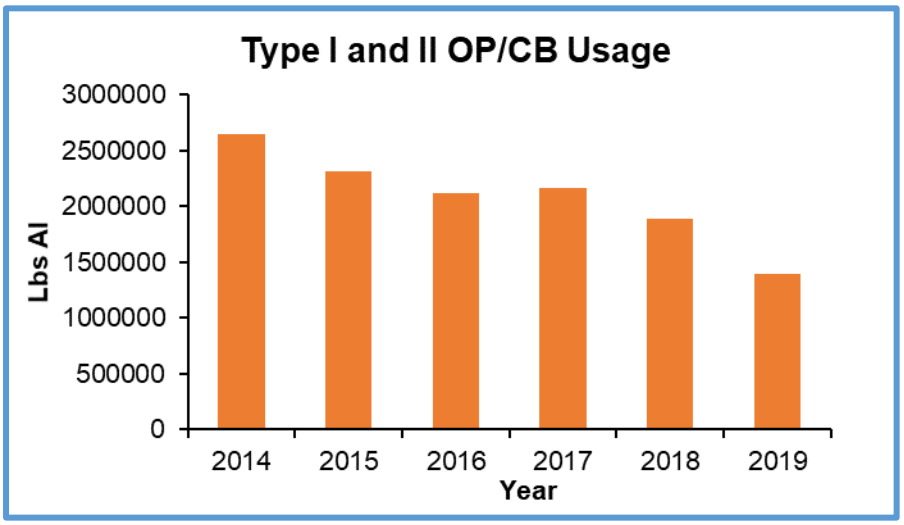


***Figure G6:** Yearly usage of Type I and II OP/CB for agricultural purpose only (lbs. AI/year) from 2014 through 2019.*

RESULTS

Individuals under the Program

As mentioned in the Methods section above, ChE tests received by DPR from reporting laboratories were not exclusively a part of the Program. Thus, many of the tests received may have be from individuals who were not workers handling Type I and II OP/CB pesticides. In order to narrow down individuals to those who might belong to the Program, specific exclusion criteria were used. Data analyzed in the current report was limited to individuals that were suspected pesticide handlers under the Program.

Overall ChE Tests Ordered during 2014–2019 Period

The 2015 Report analyzed the data for 2011–2013 period. In this update, we analyzed the data between 2014 and 2019. During this period, DPR received 148,057 tests of 23,806 individuals from the six reporting laboratories. After cleaning and applying exclusion criteria, 22,833 individuals (95.9%) and 122,917 (82.7%) tests remained in the dataset. Then, individuals with at least three tests per year (periodic testing) (9.8%, n=2,237) and those with fewer than three tests per year (without periodic testing) (90.2%, n=20,593) were separated. Data analysis conducted was limited to a subset of individuals with only a single test in a spraying season from the pool without periodic testing (75.3%, n=17,198) and individuals with periodic testing (85%, n=19,435). It should be noted that the data exclusion process may have eliminated handlers under the Program who did not have any years in which he or she received two or more tests (e.g., a handler who only received one baseline test or one follow-up test per year). As shown in Table G2, the number of tests has been relatively steady with a slight decrease in 2018–2019. The number of individuals has been steady year after year.

***Table G2**. Number of tests ordered and associated individuals before and after data cleaning.*

Year	Before cleaning		After cleaning and excluding	
	Tests	Individuals	Tests	Individuals
2014	24,937	7,146	20,233	6,027
2015	23,395	6,806	20,564	5,686
2016	27,251	8,205	23,512	7,044
2017	26,797	7,246	20,138	6,018
2018	24,360	6,809	19,576	5,686
2019	21,317	6,299	17,982	5,364

Tests Ordered by Registered Medical Supervisors during 2014–2019 Period

Between 2014 and 2019, only 10.6% of physicians who ordered ChE tests were registered medical supervisors, but they ordered 49.5% of the ChE tests deemed under the Program (n=122,917). The proportion of tests ordered by medical supervisors has increased each year between 2014 and 2018 and remained steady from 2018 to 2019 (Figure G7). This may 1) indicate the majority of tests received are from handlers under the Program, and 2) demonstrate the effectiveness of the medical supervisor registration process implemented in 2017. Medical supervisors who were identified through the aforementioned DPR survey (2015 Report) were included for the years prior to the registration process (2014–2016).

Furthermore, the analysis showed that medical supervisors ordered the majority of tests (67.8%) with 14-day baseline estimates (data not shown).

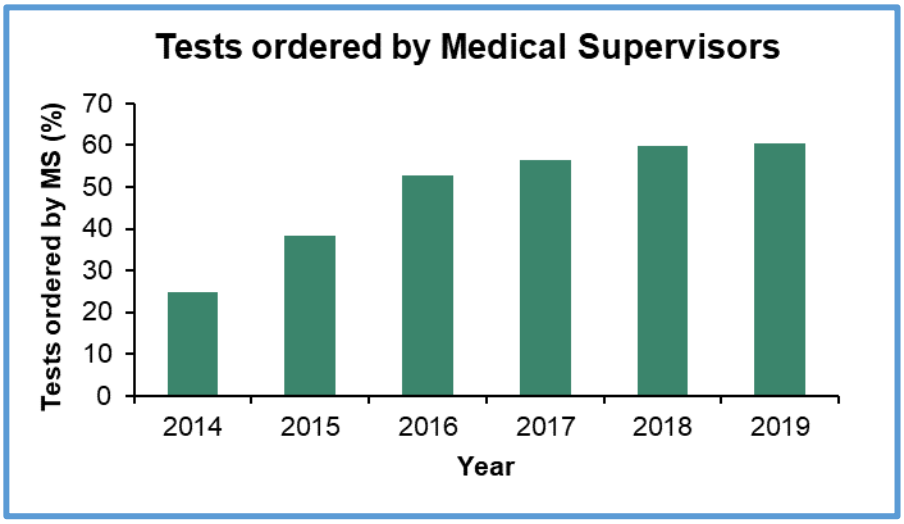


Figure G7: *Proportion of tests ordered by medical supervisors from 2014 through 2019.*

Individuals under the Program with Periodic Testing (i.e., more than 3 tests within a year)

As mentioned in the 2015 Report, work activities of handlers were often managed in order to prevent exceeding the threshold for follow-up testing (more than six days within a 30-day period). This is consistent with the current finding that the proportion of individuals with periodic testing is low (16.4%, n=2,237), while the number of tests associated with these individuals is a significant portion of overall tests (45.3%, n=43,470).

Individuals with 14-day Baseline Estimates

From 2014 through 2019, 62.5% of individuals with periodic testing (n=1,399) have identifiable 14-day baseline estimates. The proportion of individuals with these baseline estimates increased in 2015 and remained steady through 2019 (Figure G8).

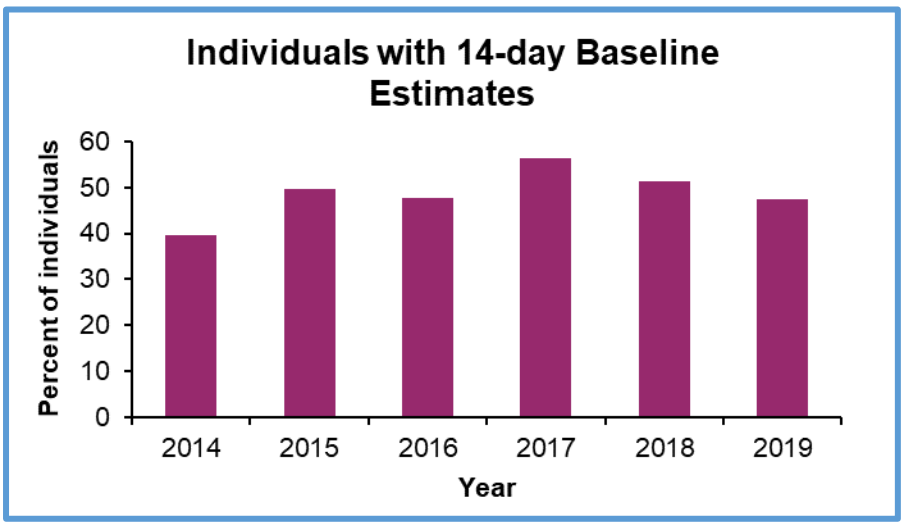


Figure G8: *Proportion of individuals with periodic testing that had 14-day baseline estimates from 2014 through 2019.*

It should be noted that from 2014 onwards, OEHHA has made several outreach efforts to physicians, including conducting in-person visits to medical supervisors and registration of medical supervisors. In each of its outreach efforts to physicians, OEHHA recommended physicians to use two tests 3 to 14 days apart to establish a ChE baseline after at least a 30-day exposure-free period. These outreach efforts may have contributed to the increase observed here in proportion of individuals with 14-day baseline estimates.

ChE Depressions requiring Action

ChE depressions were calculated for individuals with periodic testing using 14-day baseline estimates and maximum value as described in the methods section. ChE depressions over the 20% threshold were considered significant. Additionally, the Program requires employers to investigate workplace practices should any of their regular handlers meet this threshold.

Figure G9 illustrates that the number of ChE depressions have decreased in recent years (2017–2019) for individuals with 14-day baseline estimates, and maximum value baseline estimates. From 2014–2019, the pool of individuals with 14-day baseline estimates had 211 ChE tests from 133 individuals that showed significant ChE depressions (>20%). The number of individuals with significant ChE depressions have decreased since 2014, with a 68.8% decrease occurring between 2014 and 2015. This overall decrease correlates with decrease in pesticide use. There has also been an observed decrease in significant ChE depressions in the last three years, which was when the medical supervisor outreach and registration efforts were first initiated.

For individuals with maximum value baseline estimates, 480 ChE tests from 248 individuals were significantly depressed. ChE depression trends differed slightly from what was observed with individuals with 14-day baseline estimates. The proportion of significant ChE depressions for individuals with maximum value baseline estimates varied and a general decrease in ChE depressions was not observed. In addition, annual number of ChE depressions was much higher compared to the other approach using 14-day baseline estimates. This finding could suggest that the maximum value approach may have led to an overestimation of ChE depressions.

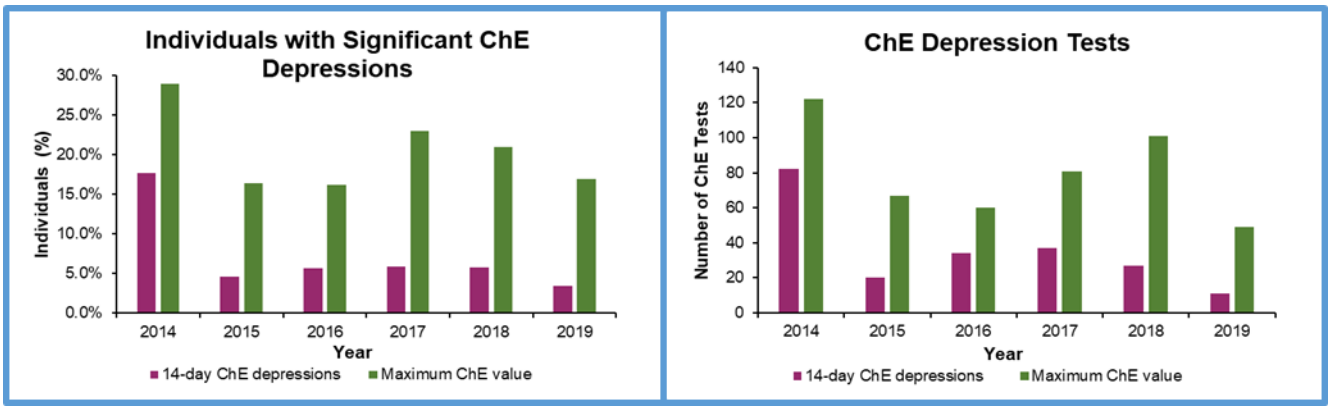


Figure G9: *Yearly proportion of individuals (left) and tests (right) with significant ChE depressions (over 20%) from 2014 through 2019.*

As seen in Figure G9, the proportion of individuals with significant ChE depressions has decreased each year over the past three years. The decrease in the proportion of individuals with significant ChE depressions could be attributed to the yearly decrease in Type I and II OP/CB usage since 2017 (Figure G6). On the contrary, usage of these pesticides has also decreased between 2014 and 2016, however the proportion of individuals with significant ChE depressions increased in those years, suggesting that the decrease observed from 2017 onwards could be attributed to other factors. In the same time period (2017–2019), the registration of medical supervisors was initiated and a yearly increase in proportion of individuals seen by those physicians has been observed (Figure G10).

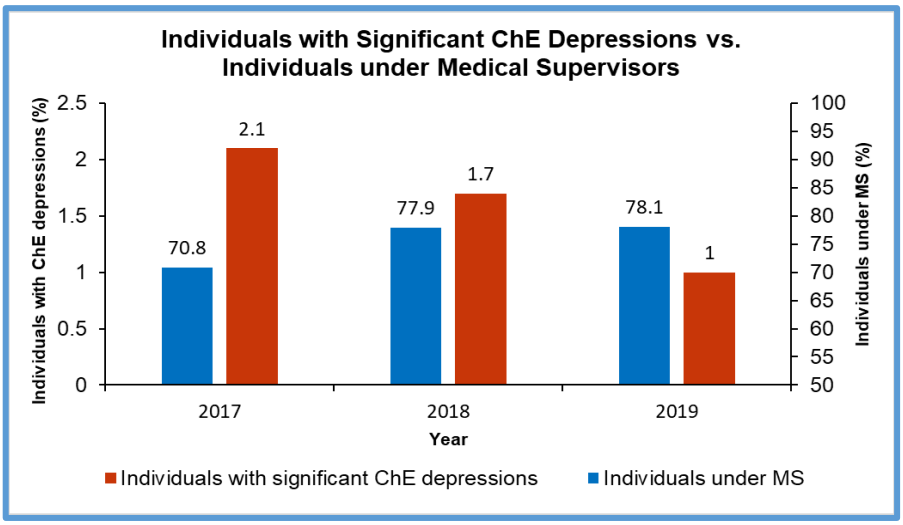


Figure G10: *The proportion of individuals under medical supervisors (MS) have increased (blue) since 2017, when the medical supervisor registration process was first initiated. In that same period, the proportion of individuals with significant ChE depressions have decreased (red).*

Perhaps medical supervisor registration, along with other interventions (i.e., DPR's grower and PCB survey and inspections from 2015–2018), contributed to the increase in the number of handlers participating in the Program and led to an increase in compliance of HSC § 105206, which led to fewer handlers being excessively exposed to Type I and II OP/CB pesticides.

Table G3: *Depression of ChE tests from individuals with 14-day baseline.*

Year	RBC Tests		Plasma Tests		Individuals	
	20-30%	>30%	20-40%	>40%	Workplace investigation threshold	Workplace removal threshold
2014	5	13	42	22	30	19
2015	0	0	19	1	16	1
2016	2	0	28	4	20	2
2017	2	0	35	0	24	0
2018	6	3	18	0	18	2
2019	1	0	9	1	9	1
Total	**16**	**16**	**151**	**28**	**117**	**25**

Table G3 shows significant depression of ChE tests from individuals with 14-day baseline estimates. Overall, from 2014–2019, 9.5% (n=133) individuals had significant ChE depressions from the total number of individuals with 14-day baseline estimates (n=1,399). Of these 9.5%, a small proportion (18.8%, n=25) had ChE depressions that exceeded the workplace removal threshold (>30% for RBC and >40% for plasma). Most individuals with significant ChE depressions (86.6%, n=117) only had a ChE depression that exceeded the workplace investigation threshold but did not require removal (20–30% for RBC and/or 20–40% plasma). Additionally, plasma ChE depressions were much more frequent than RBC ChE depressions, which was expected because plasma ChE is known to be more labile and more rapidly inactivated by pesticides so changes can be detected soon after exposure. This table also shows that the number of tests are close to the number of individuals, which suggests that most individuals were not experiencing multiple ChE depressions within a spraying season. Only 19 individuals had multiple ChE depressions within a single spraying season, with nine of the individuals experiencing significant ChE depressions across spraying seasons (data not shown). There seems to be a general trend of decrease of number of individuals with ChE depressions that exceeded the workplace removal threshold and number of individuals with ChE depression that exceeded the workplace investigation threshold but did not require removal, according to the 2014–2019 data.

Table G4 shows depression of ChE tests from individuals with baseline estimates derived from their maximum ChE value. Over the 6 year period (2014–2019), from the total number of individuals without 14-day baseline estimates (n=838), a relatively small proportion of individuals (26.2%, n=220) had ChE depressions that exceeded the workplace removal threshold (>30% for RBC and >40% for plasma). Most individuals (81.81%, n=180) only had a ChE depression that exceeded the workplace investigation threshold (20–30% for RBC and/or 20–40% plasma).

Table G4: *Depression of ChE tests from individuals with maximum value baseline.*

Year	RBC Tests		Plasma Tests		Individuals	
	20-30%	>30%	20-40%	>40%	Workplace investigation threshold	Workplace removal threshold
2014	11	14	76	21	46	15
2015	2	2	57	6	36	5
2016	1	15	41	3	29	4
2017	7	3	69	2	51	3
2018	15	29	53	4	29	11
2019	4	1	43	1	29	2
Total	**40**	**64**	**339**	**37**	**220**	**40**

Correlation Analysis

The association between pesticide-use patterns and number of ChE tests ordered was investigated in order to evaluate whether tests received by DPR were related to the Program. A positive correlation between ChE test orders and use of Type I and II OP/CB would indicate that tests received by DPR are related to the Program. As described in the Methods section, 2014–2019 PUR data was used to determine the association between the

number of ChE tests ordered and 1) monthly use of OPs/CBs and 2) the quantity of OPs/CBs used per county and area group.

The correlation between the monthly number of tests (baseline, follow-up, and depression) ordered between 2014 and 2019 and monthly pesticide usage (PUR data for Type I and II OP/CB pesticides) from 2014–2019 was determined for each area group.

Temporal Analysis of ChE Tests using the 14-day Approach

The relationship between baseline, follow-up, ChE depression, and monthly Type I and II OP/CB usage was analyzed on the area group level for two pools of individuals (those with and without 14-day baseline estimates). For individuals with 14-day baseline estimates, the temporal relationship between suspected baseline tests and monthly pesticide spraying was not analyzed because the criteria for baseline identification only included tests that occurred during low-spraying months, which, by definition, would have skewed the analysis towards a correlation.

Follow-up ChE Tests

There was a significant correlation between the number of follow-up ChE tests and Type I and II OP/CB PUR data on the area group level (Figure G11). Follow-up tests are expected to correlate with spraying patterns because such tests are required for handlers once they are regularly handling pesticides. The significant correlation between pesticide use and follow-up tests suggests that a large proportion of ChE tests being analyzed are for handlers under the Program.

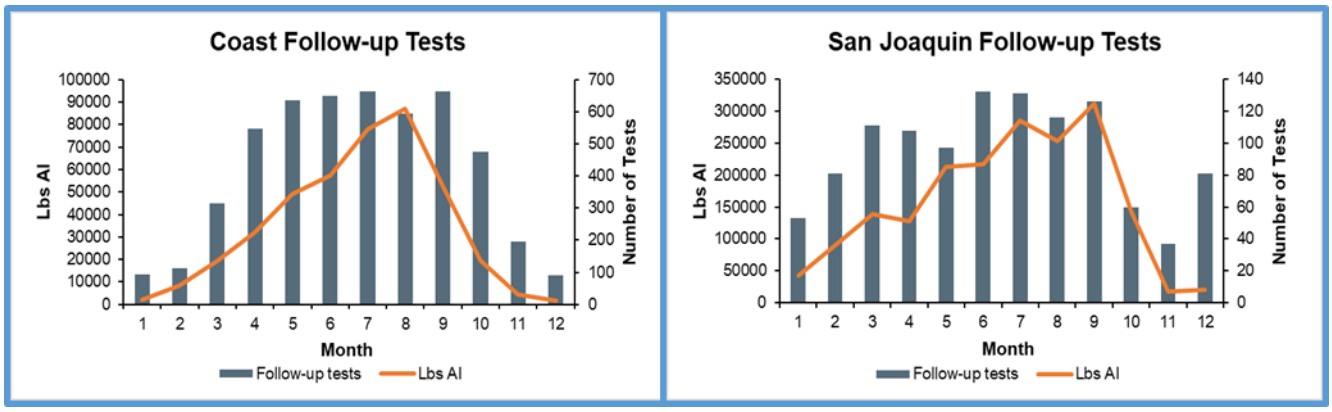


Figure G11: *Follow-up tests from Coast (left) and San Joaquin (right) area group significantly correlated with PUR data (Coast: Pearson's r = 0.87, p<0.001; San Joaquin: r = 0.82, p-value = 0.001).*

ChE Depressions and PUR

A correlation between number of significant ChE depressions (>20%) was observed when comparing ChE depressions and PUR data for the Coast area group (Figure G12). Interestingly, for the San Joaquin Valley area group, significant ChE depressions were not correlated with PUR data and tended to occur earlier in the spraying season.

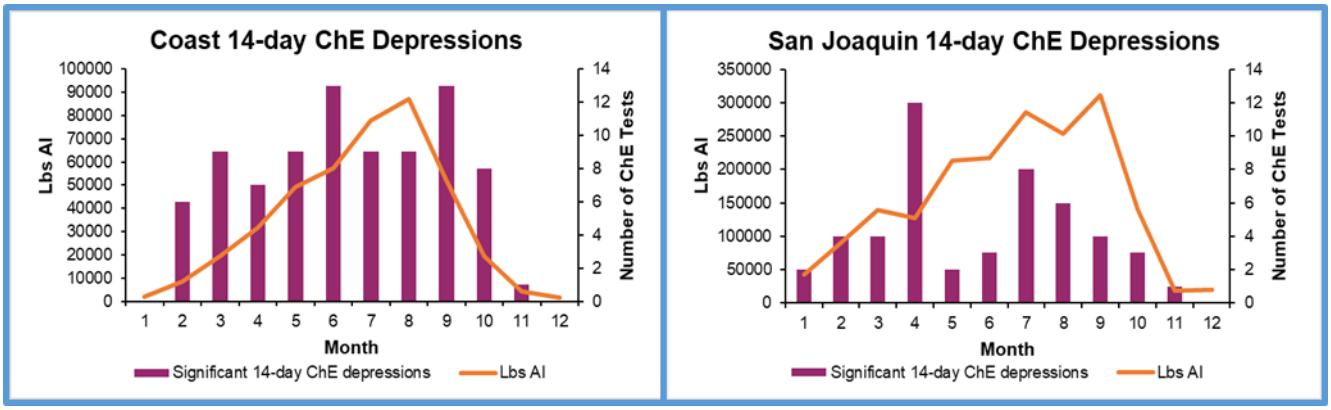


Figure G12: *ChE depressions from both Coast (left) and San Joaquin Valley area groups correlated with PUR data (Coast: Pearson's r = 0.7302, p>0.05; San Joaquin: Pearson's r = 0.45, p-value = 0.19) although it was not statistically significant for San Joaquin).*

Temporal Analysis of ChE Tests using the Maximum ChE Value Approach

For individuals without 14-day baseline estimates, maximum values were used as baselines in order to detect potential depressions.

Baselines

As expected, an inverse correlation between the monthly number of maximum baseline estimates and PUR data for each area group was observed, although not statistically significant. For Coast, the correlation was approaching significance (p = 0.08). Visual inspection of Figure G13, indicates that maximum ChE values are occurring when the spraying is lower, which may support the approach of using maximum ChE values as proxy baseline estimates.

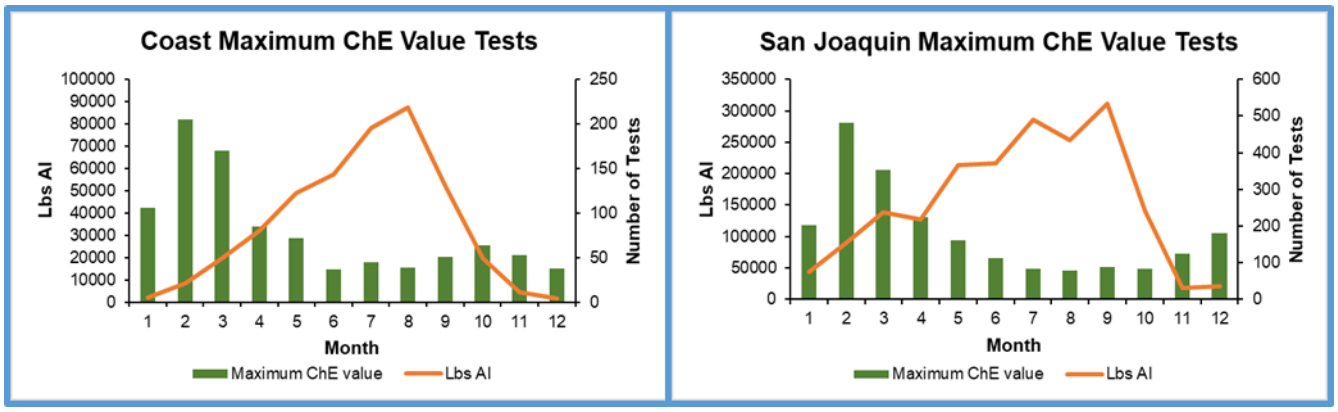


Figure G13: *Monthly maximum ChE tests and PUR data for Coast (left) and San Joaquin Valley (right) area groups were inversely correlated although not statistically significant (San Joaquin: Pearson's r = -0.43, p-value = 0.16; Coast: Pearson's r = -0.46, p = 0.13).*

Follow-up ChE Tests

Significant correlations between the number of follow-up tests and PUR data were observed (Figure G14). The positive correlation could indicate that the number of ChE tests ordered per month relate to pesticide use patterns, which may suggest a large proportion of tests that associated with this pool of individuals may be under the Program.

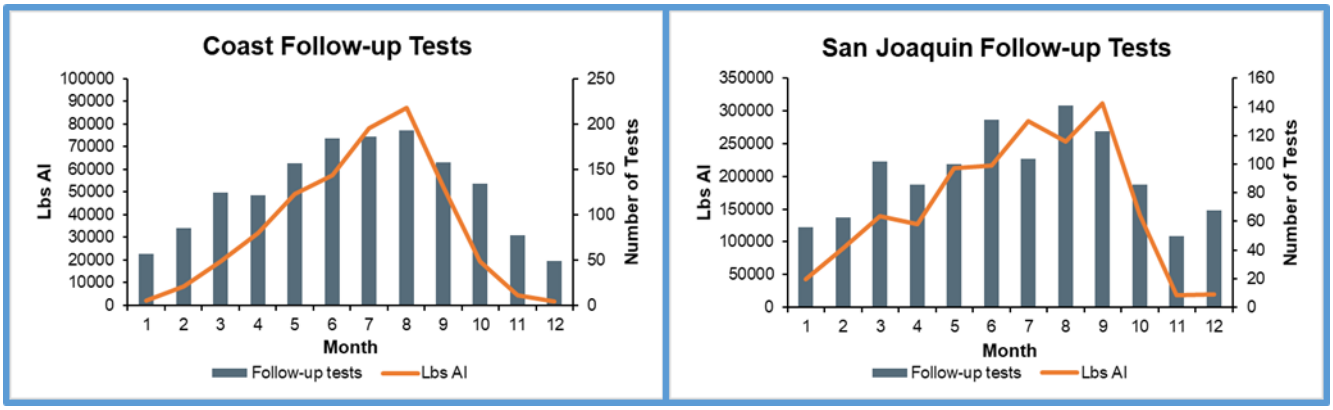


Figure G14: *Follow-up tests from Coast (left) and San Joaquin Valley (right) area groups significantly correlated with 2014–2019 PUR data (Coast: Pearson's r = 0.94, p-value = <0.001; San Joaquin: Pearson's r = 0.88 , p-value = <0.001).*

ChE Depressions from Maximum Values

ChE depressions calculated from maximum ChE values (after exclusion of outliers) were determined (Figure G15). The correlation between ChE depressions over 20% and PUR data was analyzed. In both Coast and San Joaquin Valley area groups, a positive correlation was observed between ChE depressions and OP/CB use, which may suggest that these depressions are associated with handlers under the Program. The correlations also support the approach of using maximum ChE values as proxy baseline estimates.

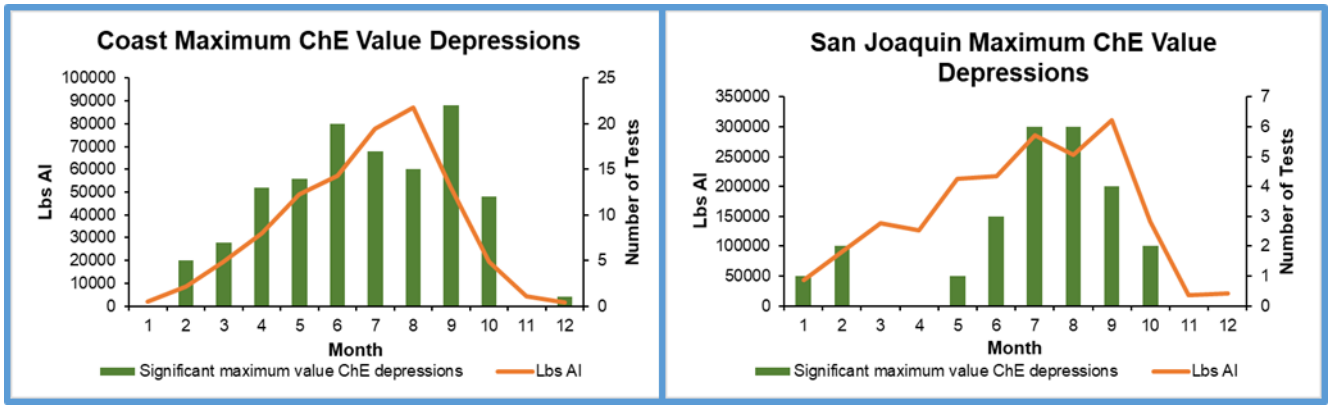


Figure G15: *ChE depression tests were significantly correlated for Coast area group (left) with Pearson's r = 0.83, p<0.001 and San Joaquin Valley area group (right) with Pearson's*

Single ChE Tests

For the San Joaquin Valley area group, an inverse temporal correlation was observed between total number of single ChE tests and average pounds of Type I and II OP/CB active ingredients from 2014–2019. This suggests that a large proportion of tests may have been baseline tests since there is high pesticide usage in most counties within this area group, thus an inverse correlation was observed for that area group (Pearson's r = -0.37, p>0.05). On the contrary, the Coast area group lacked an inverse correlation (Pearson's r = -0.02, p>0.05) which could have been, in part, due to ChE tests being ordered from low-pesticide use counties within this area group, as seen in Figure G16. Non-agricultural related ChE testing may have occurred in these counties, as the density of medical facilities in certain counties within the Coast area group is high.

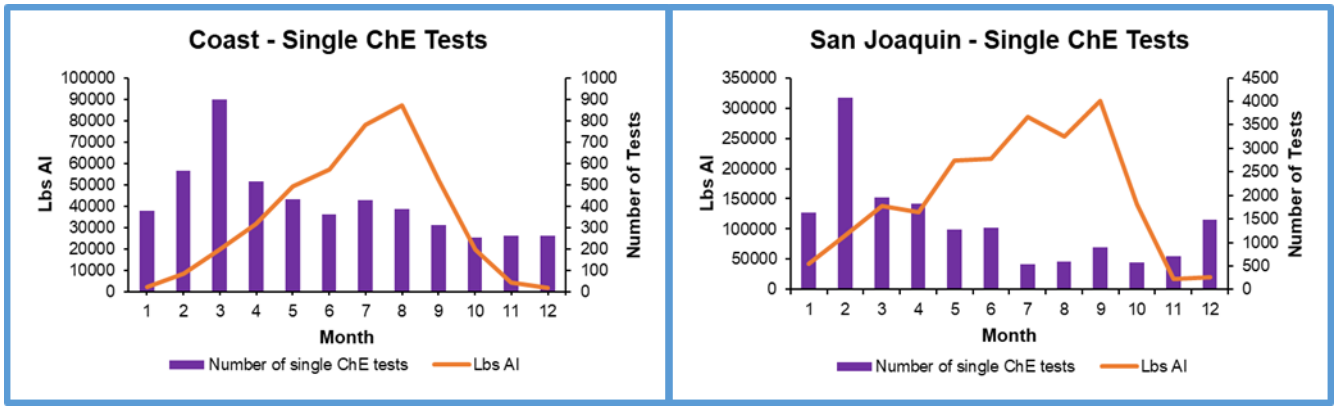


Figure G16: *The temporal correlation between number of single ChE tests ordered and PUR data from 2014–2019 from Coast (left) and San Joaquin Valley (right) area groups. An inverse correlation was observed in the San Joaquin Valley area group (Pearson's r = -0.37, p >0.05), but not for the Coast area group (Pearson's r = -0.02, p>0.05).*

Test Purpose for Single ChE Tests

The purpose of test field was analyzed in order to determine whether individuals with single ChE tests may have been part of the Program. The majority of single ChE tests were missing purpose of test (75.8%, n=34,211). Close to a quarter of ChE tests (24.2%) had a

purpose of test indicated in the field. About a third of these tests (32.7%, n=3,559) contained the term “baseline”. Single ChE from individuals who are receiving tests every year between 2014 and 2019 could be baseline tests under the Program. However, data analysis showed that less than one percent of individuals (0.8%, n=94) were tested every year, and only three tests from those individuals contained “baseline” in the test purpose field. As expected, few tests contained the term “follow-up” (3.5%, n=384) and only two ChE tests contained the term “recovery”. It should be noted that individuals with tests containing “follow-up” or “recovery” could have obtained baseline tests during previous spraying seasons, however the data analysis conducted for this report was limited to each spraying season.

Temporal Analysis of Purpose of Test

In order to evaluate the accuracy of the test purpose terms used and the relevancy of those tests to the Program, a correlation analysis on when tests with specific test purpose terms were ordered and monthly average PUR data from 2014–2019 was conducted. Test purpose terms that may indicate baseline, follow-up, and recovery tests under the Program are shown in Table 2.

A temporal correlation analysis between the total number of tests with test purpose terms indicating baseline, follow-up, and recovery and monthly average PUR data from 2014–2019 was conducted (Figure G17). As expected, an inverse correlation between the number of tests with a baseline test purpose and PUR data was observed for both Coast and San Joaquin area groups. A positive correlation between the number of tests with a follow-up purpose of test and PUR data was observed for the Coast area group, but not for San Joaquin. The lack of correlation observed for San Joaquin may be due to lower numbers of tests with a follow-up purpose of test in this area group. Only twelve tests indicated recovery and were all from the San Joaquin area group. These findings suggest tests containing test purpose terms that may indicate baseline, follow-up, and recovery (see Table 2) may pertain to the Program and are correctly being used by medical supervisors.

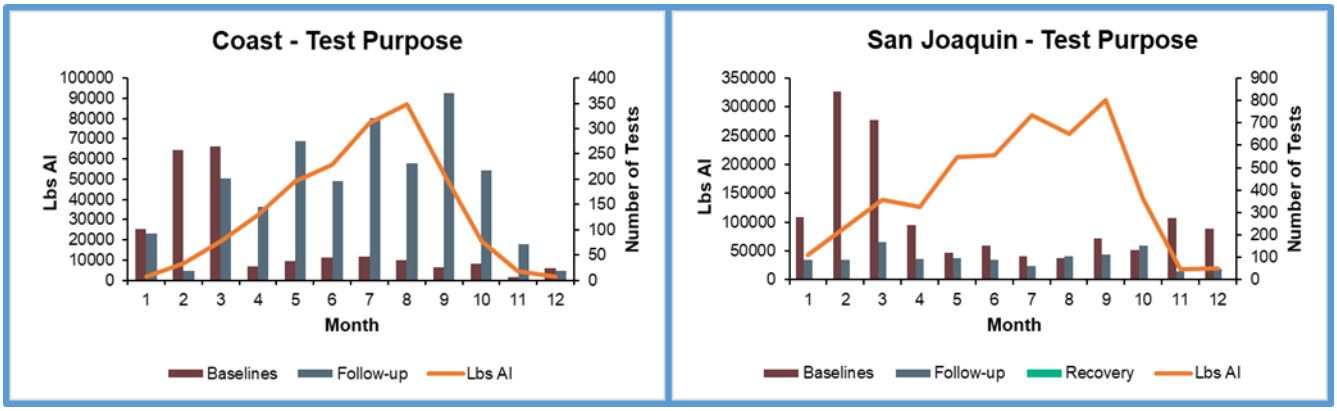


Figure G17: *An inverse correlation between monthly average 2014–2019 PUR data and total number of tests with a purpose of test that indicated a baseline test was observed for both Coast (left) and San Joaquin Valley (right) area groups (Coast: Pearson’s r = -0.31, p>0.05; San Joaquin: Pearson’s r = -0.39, p>0.05) . A positive correlation between tests with purpose of test that indicate a follow-up test and PUR data was observed for the Coast area group, but not for San Joaquin Valley (Coast: Pearson’s r = 0.77, p <0.05; San Joaquin: Pearson’s r = 0.28, p >0.05).*

Supplemental Study II:
Analysis of ChE Trends of Individuals with "Recovery" Tests

The temporal patterns of the ChE activity levels of individuals (n=5) associated with "recovery" tests (n=12) were analyzed. Figure G18 shows an example of individual's plasma ChE tests over time compared to the PUR data for Fresno County, where the ChE tests were ordered. All ChE tests that indicated recovery were preceded by tests that contained "baseline" or "follow-up" in the test purpose field. The trends of plasma ChE activity levels over time for all individuals represented non-monotonic curves that showed significant depressions (≥20–40%) following a single test or two tests taken 3–14 days apart, then a gradual increase. Some ChE activity levels of individuals recovered to levels within 80% of the initial test(s) after depressions. These observations reflect workers under the Program whose ChE activity levels exceeded the workplace removal action threshold thus removed from work, elucidating the gradual recovery to levels within 80% of their single or 14-day baseline test(s).

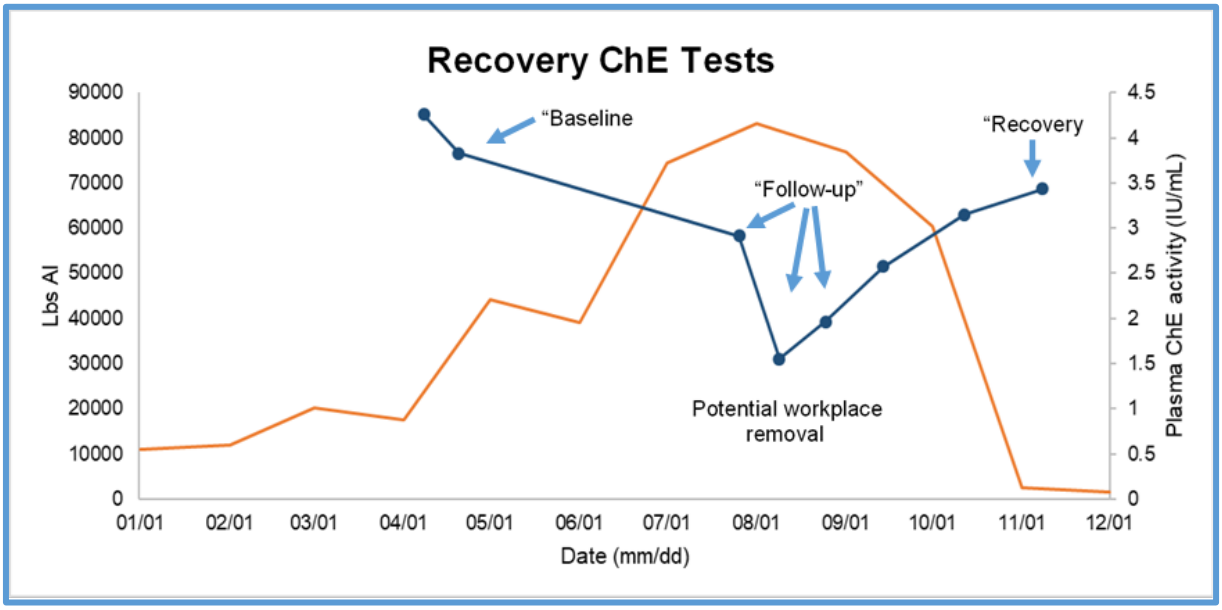


Figure G18. *An individual's plasma ChE tests over time (dark blue). The labels in quotations are the test purpose associated with each test, indicated by the arrows. There was a significant depression between July and August, which could have been due to excessive pesticide exposure. Workplace removal may have occurred in August, since ChE activity levels improved afterwards. The last test within the spraying season was within 80% of the baseline level, which was labeled "recovery". This trend was compared to the PUR data for Fresno County, where the ChE tests were ordered.*

Geospatial Analysis

Geospatial analysis was done to determine the association between the number of ChE tests ordered and ChE depressions detected and pounds of Type I and II OP/CB active ingredients used for each county. This analysis was conducted on the county level, instead of the area group level, in order to accurately determine a correlation. Since there are only five area groups for the entire state, the area group level would mask correlations that could be seen on a smaller level.

Number of ChE Tests per County

There was a signficant correlation between the number of ChE tests ordered and the pounds of Type I and II OP/CB active ingredients used for each county (Figure G19). This finding suggests that a significant proportion of ChE tests after data cleaning and applying the exclusion criteria may have indeed been related to the Program.

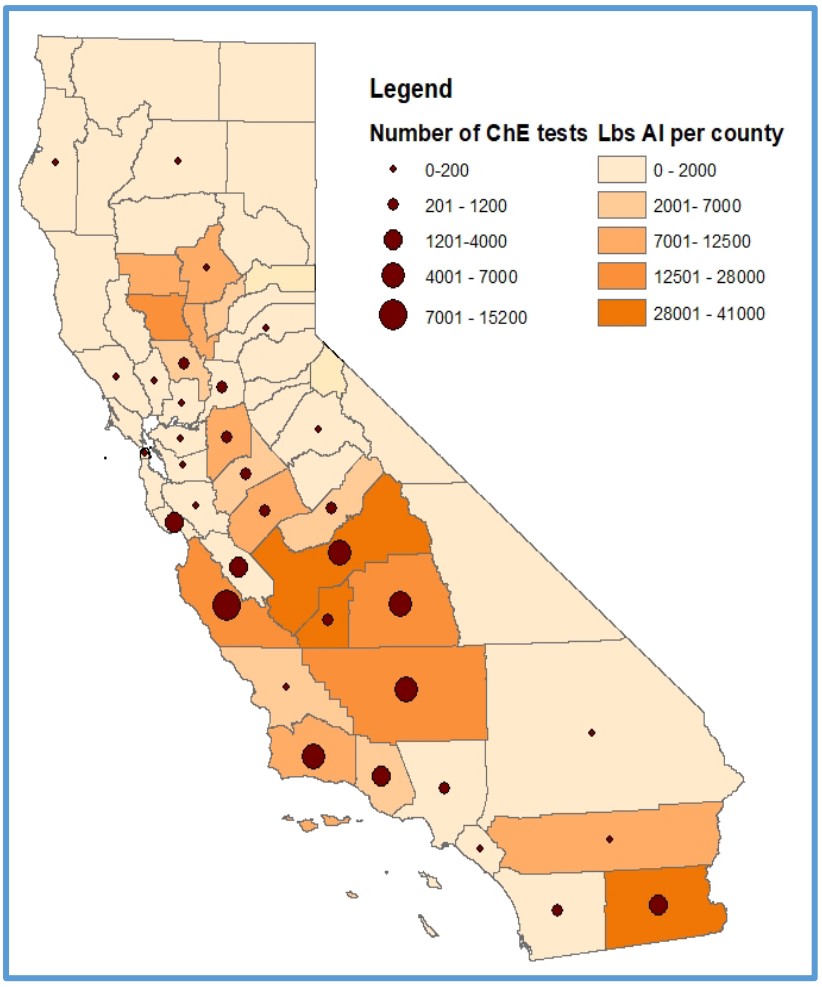


Figure G19: *Geographic distribution of Types I and II OP/CB pesticides and number of ChE tests by county across California (2014–2019). A significant correlation was determined between total number of ChE tests and average Type I and II OP/CB active ingredients used per county (Pearson's r = 0.39, p-value <0.05).*

Similar to what was observed in the 2015 Report, geographic analysis revealed that in several California counties the OP/CB use did not correspond with the number of ChE tests received. In the 2015 Report, several explanations were offered some of which were verified. These include: Several counties that had relatively high OP/CB use (e.g., northern Sacramento Valley) had very few ChE test results. A lack of test results from these counties might be due to: 1) missing location information on the ChE test reports, 2) employee's worksite and physician's location in adjacent counties, 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. Other counties with no or very low pesticides use (e.g., San Francisco) had disproportionally high number of tests. These tests were most likely from

individuals not participating in the Program (e.g., pre-operative testing, Alzheimer's drug monitoring, liver disease screening, and aging research studies).

Number of ChE Depressions per County

Similarly, ChE depressions derived from the 14-day baseline estimates and maximum ChE values were also significantly correlated with the amount of OPs/CBs used per county (Figure G20).

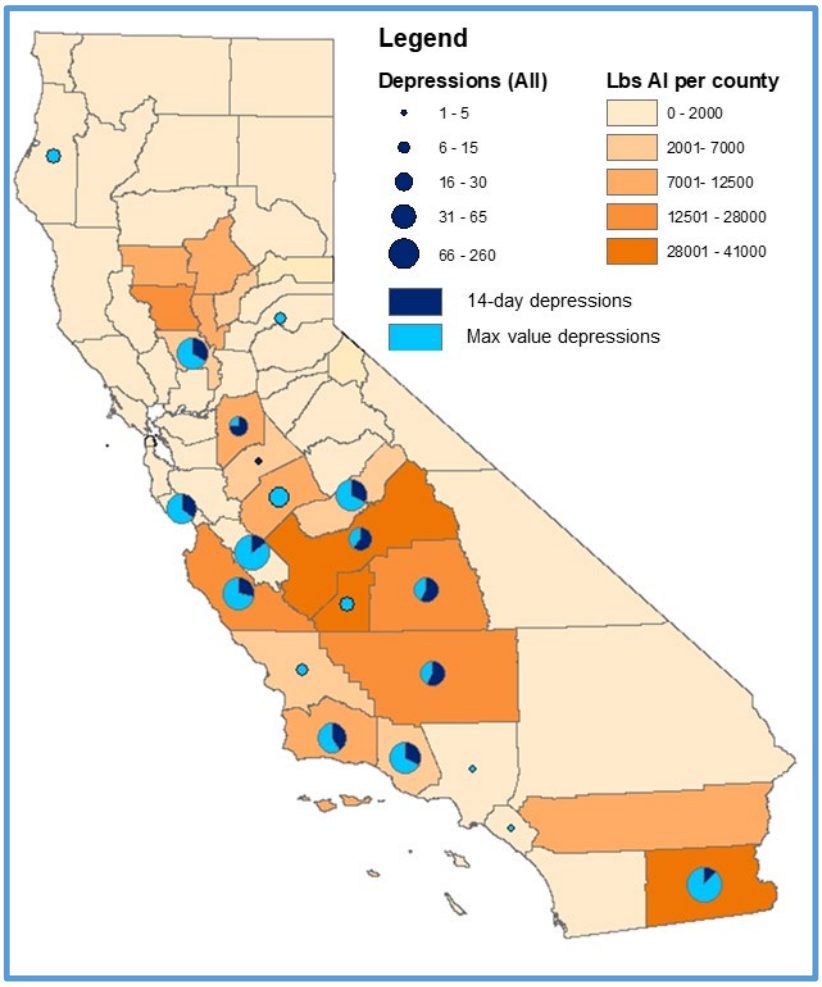


Figure G20: *Geographic distribution of Type I and II OP/CB pesticides and number of ChE depressions by county across California (2014–2019). There was a significant correlation between estimated significant ChE depressions and poundage of active ingredients used per county (Pearson's r = 0.38, p-value <0.05). In most counties, there were more maximum ChE value depressions (light blue) than 14-day depressions (dark blue). Some depressions were observed in counties that did not have high Type I and II OP/CB usage, but were adjacent or near counties that did.*

Spatial Analysis of Single ChE Tests

The number of ChE tests from individuals without periodic testing (single ChE tests ordered per individual per spraying season) significantly correlated with the amount of Type I and II OP/CB active ingredients used per county (Figure G21). The correlation was slightly weaker compared to the correlation between ChE tests from individuals with periodic testing, which was expected. Additionally, some counties with low pesticide use, especially in the Coast area group (e.g., Alameda, San Francisco), had a high number of single ChE tests ordered. These observations suggest that although a large number of individuals with single ChE tests were a part of the Program, a significant proportion may have not been.

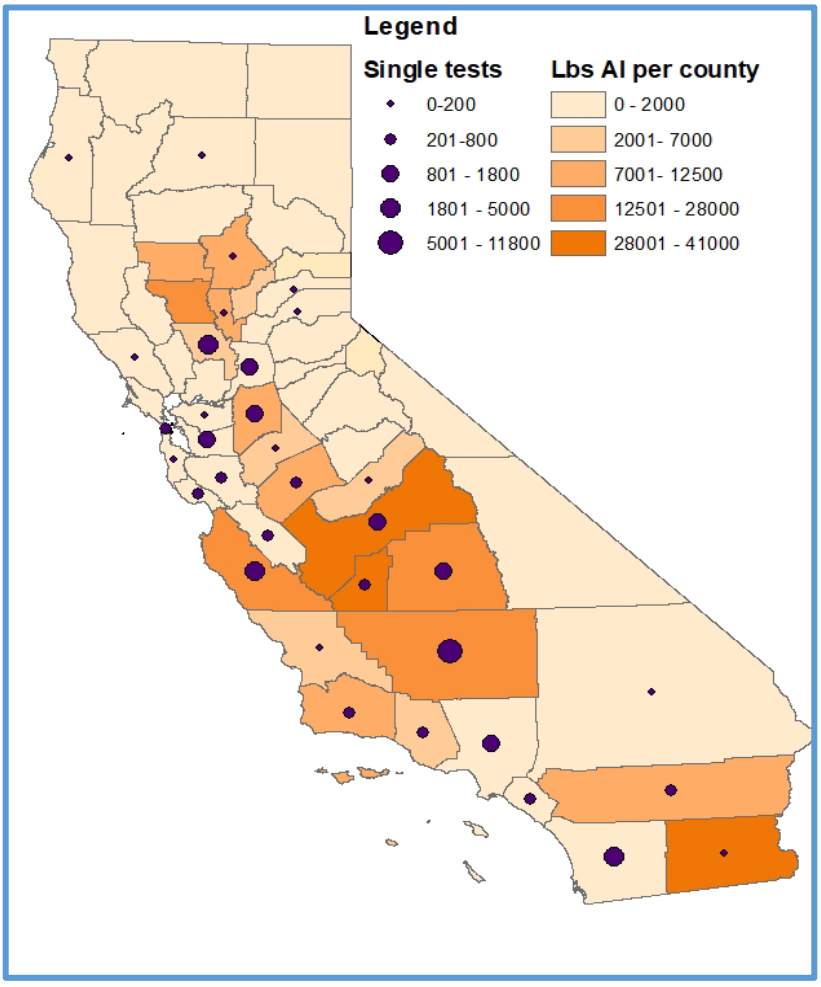


Figure G21: *Total number of single ChE tests per county. There was a significant spatial correlation observed between the number of tests and average pounds of Type I and II OP/CB active ingredients used per county between 2014 to 2019 (Pearson's r = 0.33, p = 0.05).*

SUMMARY OF FINDINGS

Consistent with the 2015 Report, laboratory-based electronically-reported ChE data is a useful tool for evaluating the effectiveness of the Program. Changes made since the 2015 Report allowed OEHHA and DPR to not only assess the implementation of the Program on a statewide-level, but also regionally. Using this data, compliance of employers and medical supervisors under the Program were assessed by looking for patterns in 1) the spatial and

temporal distributions of ChE tests and 2) the number of individuals with repeated ChE depressions within spraying seasons. Temporal analysis showed significant correlation between ChE depression and follow-up ChE tests and monthly Type I and II OP/CB usage, while an inverse correlation was observed for maximum ChE values (potential baseline estimates). This suggests that handlers were receiving baseline and follow-up ChE tests from medical supervisors during the correct periods of spraying seasons, following HSC § 105206 requirements. Spatial data analysis revealed that the number of ChE tests and depressions were significantly correlated with the distribution of Type I and II OP/CB usage on a county-by-county basis, which suggests that there is high participation from handlers from areas with higher Type I and II OP/CB I usage, corroborating the conclusion made in the 2015 Report. Using the electronically-reported ChE data, OEHHA and DPR were also able to evaluate whether workers under the Program were protected from excessive exposure to Type I and II OP/CB as well as the effectiveness of the Program. Analysis of ChE data showed that the number of depression tests and individuals with significant ChE depressions have steadily decreased over the past three years (2017–2019). Furthermore, only a small proportion of individuals had multiple ChE depressions within a spraying season and across multiple spraying seasons. Additionally, a small percentage of individuals exceeded the workplace removal threshold. These findings indicate that the Program is effective in protecting workers who regularly handle Type I and II OP/CB pesticides.

One limitation from the 2015 Report that remained not addressed in the current report was the inability to determine the total number of handlers who qualify for the Program by using the laboratory-based electronically-reported ChE data. Similar to the 2015 Report, the data quality received by DPR from the reporting laboratories was poor. This prompted extensive data cleaning and excluding, which could have erroneously excluded tests and handlers from the data. Further improvements in data collection and reporting from medical supervisors and laboratory personnel could help with the accuracy of the data analysis in the future.