

## 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 3CCR §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 4° 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 20° 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 25° 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 3CCR §6728 (c)(2)(A) shall be conducted by the same laboratory method.

(8) 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.