IV. ENVIRONMENTAL DATA AND BIOLOGIC EVALUATION

Environmental Data

Airborne concentrations of carbaryl and the potential for respiratory and dermal exposures have been examined in the workplace. The three major types of possible occupational exposure--the manufacture of technical carbaryl, the production of formulations of carbaryl, and the use of carbaryl for application purposes--have been considered.

Best and Murray [28] reported air concentrations at various sites in a carbaryl-manufacturing plant. The air samples were collected on membrane filters and analyzed by a colorimetric method for determining the airborne concentration of carbaryl. The mean carbaryl concentration of 49 air samples in the production area was 0.23 (range 0.03-0.73) mg/cu m of air. In the bagging area, 18 air samples with a mean of 0.75 (range 0.20-1.60) mg/cu m were collected under "normal conditions." Under abnormal conditions (described in Chapter III), the carbaryl concentration ranged from 19.0 to 40.0 mg/cu m, with an average of 29 mg/cu m for six samples. "Stackers" (handlers of bags for shipment) were exposed at a mean air concentration of 0.64 (range 0.05-1.52) mg/cu m based on six samples collected. In the air separator house, only two airborne concentrations of carbaryl were measured, and these were in the range of 29.0-34.0 mg/cu m with an average of 31.0 mg/cu m. Union Carbide Corporation [13 (sec 9,10)] also supplied information on individual air concentration samples from the shipping department of the same plant. These air samples, collected and analyzed in the same manner as that used by Best and Murray, [28] were obtained sporadically over a 2.5-year period. Mean values, presented

below, are calculations from the original data. A total of 96 air samples from the bagging operation had a mean carbaryl airborne concentration of 2.96 mg/cu m, with a range of 0.32-13.33 mg/cu m. Near an instrument panel, the mean airborne concentration was 2.76 (range 0.12-10.51) mg/cu m based on 44 samples. In the shipping department office, the mean carbaryl airborne concentration was 0.33 (range 0.04-0.83) mg/cu m from 43 samples, which was comparable to the mean airborne concentration in the outside loading area of 0.37 (range 0.04-0.74, 8 samples) mg/cu m. Mean airborne carbaryl concentrations in railroad cars and trailer trucks, presumably loaded with bags of carbaryl, were 0.54 (range 0.10-1.22, 24 samples) and 0.64 (range 0.26-1.62, 9 samples) mg/cu m, respectively.

Comer et al [88] investigated potential dermal and respiratory exposures to carbaryl in formulating-plant workers and orchard spray workers. The subjects included baggers and mixers at three plants formulating 4 and 5% carbaryl dust and spray workers operating tractor-drawn airblast equipment for applying 0.045-0.06% carbaryl spray, apparently from a wettable powder, to fruit trees. Dermal contamination by carbaryl sprays and dusts was determined by attaching absorbent cellulose pads and layered gauze pads, respectively, to various areas of the workers' bodies and clothing. Respiratory exposures were estimated from the contamination on special filter pads inserted in the filter cartridges of respirators worn by the subjects. The filter pads were shielded to prevent direct impingement of droplets or particles onto the pads except for those carried by respiratory action. A total of 480 dermal and 73 respirator-pad samples were analyzed spectrofluorometrically for carbaryl during different exposure situations including 48 for formulating-plant workers and 32 for

orchard spray workers. In addition, the authors measured the urinary excretion of 1-naphthol in 102 urine samples from the formulating-plant workers, using a spectrofluorometric method. Mean values for potential carbaryl exposure among the formulating-plant workers were calculated to be 73.9 (range 0.80-1,209) mg/hour of work activity by the dermal route and 1.1 (range 0.03-4.1) mg/hour by the respiratory route. For the orchard workers, the mean values were 59.0 (range 1.7-212) and 0.09 (range 0.01-1.08) mg/hour by the dermal and respiratory routes, respectively. The authors found that, in the bagging and mixing sections of formulating plants, the areas with greatest potential of dermal exposure were the front of the chest and neck, the forearms and hands, and the face. Among spray applicators, the areas of greatest potential exposure were the shoulders and the back of the neck. The authors further calculated that the mean potential for both dermal and respiratory exposures of formulating workers would be 75 mg/hour, or 600 mg over an 8-hour workday. The authors did not report the airborne concentrations of carbaryl dust to which the workers in the formulating plants or orchards were exposed. In the 102 urinary specimens analyzed during and after exposure, 1-naphthol concentrations were 0.2-65.0 ppm (20-6,500 μg/100 ml), with a mean value of 8.9 ppm (890 μg/100 ml). The authors stated that carbaryl exposure at the beginning of the workday was followed by peak excretory levels of 1-naphthol by late afternoon and evening, followed by a drop to lower levels at the start of the next workday. The rate of excretion of 1-naphthol varied from 0.004 to 3.4 mg/hour, with a mean value of 0.5 mg/hour. The authors indicated that this was equivalent to the excretion of approximately 0.7 mg/hour of carbaryl, or an absorption of 5.6 mg of carbaryl during an 8-hour period.

When this absorption value of carbaryl (5.6 mg/8 hours) was compared to the total potential dermal and respiratory exposure value (600 mg/8 hours) for the formulating-plant workers, it was suggested that absorption from dermal contact with carbaryl dust was probably not very complete. The authors concluded that dermal absorption of carbaryl from dry formulations in formulating plants may be only a small fraction of the total potential calculated exposure.

Jegier [89] measured respiratory and dermal exposures to carbaryl (Sevin 50 WP, 0.5-8.0 lb/100 gal water) for tractor operators engaged in orchard-spraying operations. Air samples were collected from the tractor operators' breathing zones, and results of the analyses for carbaryl were used to calculate respiratory exposures which were determined by a filter-pad method. Filter pads were attached to double-unit respirators and absorbent materials were strapped to the forehead and wrists of observers riding beside the tractor drivers to estimate respiratory and dermal exposures, respectively. The airborne concentrations of carbaryl in seven breathing-zone samples ranged from 0.18 to 0.81 mg/cu m, with a mean of 0.60 mg/cu m. Calculated mean respiratory exposure based on the measured air concentrations and an assumed lung ventilation rate of 444 liters/hour was in close agreement with the mean respiratory exposure of 0.29 (range 0.24-0.53) mg/hour as determined by the filter-pad technique. The mean dermal exposure was estimated at 25.3 (range 18.5-30.3) mg/hour.

Simpson [90] measured dermal and respiratory exposure of workers spraying carbaryl in an orchard. Filter papers were pinned to the operators' clothing and cotton absorbent pads were inserted into the cartridges of respirators. These were analyzed for carbaryl to determine

dermal and respiratory exposures. Simpson [90] estimated the mean respiratory exposure rate to carbaryl at 482 (range 10-1,080) μg/hour. The mean dermal exposure rates, in μg/100 sq cm/hour, were: hat (or head), 1,150; arm, 1,134; chest, 1,122; shoulder, 957; back, 774; wrist, 734; and thigh, 587.

Kale and Dangwal [91] investigated the air concentrations of carbaryl and other pesticides in several agricultural settings. Air samples were collected with a midget impinger containing either distilled water or alcohol. The authors stated that the samples were collected as close as possible to a worker's breathing level and in a manner to avoid interfering with his work. In the first instance, a 50% wettable powder of carbaryl was applied to mango trees with a hand pump sprayer under medium air-movement conditions; only traces of carbaryl were detected. In the second agricultural application, 10% carbaryl dust was applied to cotton with a rotary hand duster in medium wind conditions; again, only traces of carbaryl were present. In a third farming operation, similar to the second but with high air movement, carbaryl concentrations were 0.1-0.8 mg/cu m of air. During the application in medium wind of 10% carbaryl dust by rotary hand duster to wheat approximately 30 cm high, airborne concentrations of carbaryl were 0.8-1.6 mg/cu m. Neither the collecting medium nor the analytical procedure for determining carbaryl was identified by the authors, nor did they mention any biologic evaluation of the workers.

Control of Exposure

Engineering design and work practices for carbaryl should have, as their main objectives, controlling airborne concentrations and minimizing

skin and eye contact. The environmental data previously presented suggest that concentrations of airborne carbaryl are significantly lower, and therefore there is potentially less absorption in agricultural occupations than in manufacturing and formulating operations.

In manufacturing and formulating plants and in other locations where suitable and practical, closed systems, properly operated and maintained, should be used to achieve reduced airborne concentration and to minimize skin contact. Where closed systems are not feasible, well-designed local exhaust ventilation should be provided. Guidance for design can be found in Industrial Ventilation--A Manual of Recommended Practice, [92] or more recent revisions, and in Fundamentals Governing the Design and Operation of Local Exhaust Systems, Z9.2-1971. [93] Exhaust air should not be recirculated and should be filtered to prevent pollution of the outdoor air. Neither respiratory protective equipment nor personal protective clothing is an acceptable substitute for proper engineering controls, but both should be available for emergency purposes and for nonroutine maintenance and repair situations.

Tests performed by Union Carbide Corporation [13 (sec 16)] have shown that dust clouds of technical carbaryl and formulations of carbaryl can be ignited in closed spaces, and that explosions could occur. According to this manufacturer, carbaryl also may have an electrostatic potential that could produce a spark. Thus, it is recommended that equipment in confined spaces always be adequately grounded. Union Carbide Corporation reported that the minimum concentration of technical grade carbaryl dust to produce explosive mixtures is 20.3 g/cu m.

Sampling and Analysis

In 1962, Best and Murray [28] reported measurements of airborne concentrations of carbaryl dust in a manufacturing plant, based on a sampling method using cellulose ester membrane filters (AA white grid). During subsequent determinations of airborne carbaryl dust concentrations in this plant, 0.8-μm membrane filters composed of a copolymer of acrylonitrile and polyvinyl chloride were used for carbaryl collection. [13 (sec 11)]

Popendorf et al [94] used membrane-filter air samplers (with a sampling rate of 3 ± 0.25 liters/minute) in the measurement of concentrations of foliar pesticide residues likely to become airborne in work situations. Although the use of carbaryl was not included in this report, it appears that this sampling procedure, when coupled with an appropriate analytical procedure, may be useful for evaluating airborne carbaryl concentrations.

Air sampling specific for carbaryl was reported in a study by Klisenko [95] in 1965. He used FPP-KhA filters (no other identification of material) to collect carbaryl in air for a period of 4-5 minutes at a sampling rate of 5 liters/minute. The reagent diazobenzenesulfonic acid was used to develop color with carbaryl in the methanol extract for subsequent colorimetric determination. The sensitivity was 1 mg carbaryl in 4 ml of final solution. The author did not report the efficiency of the sampling method.

In the same study, Klisenko [95] investigated carbaryl analysis by ultraviolet spectrophotometry, still using diazobenzenesulfonic acid as the coupling reagent. He found appreciable interference at 281 nm from

methanolic extracts of the FPP-KhA filters. To avoid such interference in the ultraviolet range, carbaryl in the air was absorbed at a sampling rate of 0.5 liter/minute in 5 ml of methanol in a scrubber with a porous disc. No information regarding carbaryl collection efficiency was given. The sensitivity was reported to be 0.1 μg of carbaryl in 2.5 ml of solution.

In 1971, Kale and Dangwal [91] used midget impingers (with a collecting medium such as distilled water or alcohol) for air sampling of several different types of pesticides, including carbaryl, at spraying or dusting sites. The absorbing solution selected for carbaryl collection was not identified, and no information was presented on collection efficiency or sampling details such as flowrate and sampling time. Also, the authors did not mention the analytical procedure used for the carbaryl determinations.

Reports of the filter collection methods, [13 (sec 11),28,95] as well as the scrubber methods, including midget impingers, [91,95] did not specify carbaryl collection efficiencies. Since carbaryl formulations include wettable powders, dusts, and granules, [13 (sec 18)] the membrane filter collection technique would be expected to provide an effective collection method for airborne particulate carbaryl.

Most reported environmental exposure data for airborne carbaryl dust levels have been based on the membrane-filter collection method. [13 (sec 11),28] Membrane filters which have been used for particulate collection are polymeric (plastic) [96] or glass-fiber filters. [97] Because they are highly efficient, involve no fragile glassware such as impingers, and require no liquids, membrane filters are better suited to personal monitoring than are sampling techniques utilizing glassware and liquids.

The membrane filter method is recommended as the air sampling method of choice.

The sampling method recommended in Appendix I involves the use of a glass-fiber membrane filter (Type A), 37 mm in diameter, mounted in a two-piece cassette filter holder and held in place by a backup pad. [97] Details of membrane filter sampling and airflow calibration procedures are given in Appendix I.

The analytical procedures usually employed for determining carbaryl concentrations are based on quantifying 1-naphthol [13 (sec 11),28,98-101] derived from the alkaline hydrolysis of carbaryl. In 1962, Best and Murray [28] presented a colorimetric method to quantitatively determine 1-naphthol. This method involved measurement of the intensity of a blue dye formed by the reaction between p-nitrobenzenediazonium fluoroborate and 1-naphthol. This method may be used to determine either the free urinary 1-naphthol concentration or the total urinary 1-naphthol concentration. In the former case, the metabolite is extracted from the urine, and in the latter case, the naphthol conjugates are first hydrolyzed to 1-naphthol. This method was modified by Johnson [98] in 1964 with regard to sample preparation of food products and was adopted the same year by the Association of Official Analytical Chemists (AOAC) [99] as the recommended method for the quantitative determination of carbaryl. Except for the incorporation of a further optional modification in sample preparation by Benson and Finocchiaro [100] in 1965, this method remains the official AOAC analytical method. [101]

Another analytical approach was investigated by Frei et al. [102] When carbaryl was hydrolyzed to 1-naphthol and the latter determined

fluorometrically on thin-layer chromatograms, a visual detection limit of 0.006 μg/spot and an instrumental detection limit of 0.001 μg/spot were reported. This procedure was developed for carbaryl residue analysis but was not applied to air-sample analysis.

Early attempts to use gas-liquid chromatography for the quantitative determination of carbaryl indicated that, under the conditions commonly used for such analyses, carbaryl underwent thermal decomposition to 1-naphthol. [103,104] Such analytical techniques, therefore, were not applicable to the analysis of samples which might contain 1-naphthol or carbaryl analogs that in turn might generate 1-naphthol upon thermal decomposition. Two approaches to overcoming the problem of thermal decomposition of carbaryl have been investigated.

The first approach was to prepare thermally stable derivatives of carbaryl. Fishbein and Zielinski [103] prepared trimethylsilyl carbaryl by the reaction of carbaryl with excess hexamethyldisilazane and trimethylchlorosilane in pyridine. The reaction mixture was analyzed for the trimethylsilyl derivative of carbaryl using 6-ft x 6-mm ID glass columns containing various solid supports and stationary liquid phases in a gas chromatograph equipped with a flame ionization detector. The authors did not specify the sensitivity of the method. Khalifa and Mumma [105] prepared trifluoroacetyl and heptafluorobutyryl derivatives of carbaryl, which were thermally stable under the conditions selected, for analysis by a gas chromatograph equipped with a 63Ni electron capture detector. The authors did not report the specific conditions used for derivatization, nor did they present evidence that derivative formation was quantitative. Tilden and Van Middelem [106] described a gas-liquid chromatographic method

in which the carbaryl was quantitatively hydrolyzed to yield methylamine through acid hydrolysis. The resulting methylamine was quantitatively converted to 4-bromo-N-methylbenzamide by reaction with 4-bromobenzoyl chloride. Analysis of the 4-bromo-N-methylbenzamide was performed using a gas chromatograph equipped with a 63Ni electron capture detector and a 6-ft x 4-mm glass column containing 3% Carbowax 20M on Chromosorb W. The lower limit of detection of pure carbaryl was 20 pg by this method. The use of derivatization for carbaryl analysis has been applied primarily to residue analysis, where the carbaryl is usually extracted in an organic solvent and high sensitivity is required because of the small sample sizes. Reports in which such methods have been applied to air samples have not been found, but the time-consuming stepwise handling of samples required by these methods is a distinct disadvantage.

A second approach to overcoming the problem of thermal decomposition of carbaryl during analysis by gas-liquid chromatography was to design the system so that decomposition is minimized or eliminated. Thus, Riva and Carisano [107] indicated that the recovery of undecomposed carbaryl was better than 90% for a 300-ng sample if the 0.5% SE-30 on 100-120 mesh Gas-Chrom P column was silanized by repeated injections of hexamethyldisilazane before sample injection. Presumably the silane treatment prevents the adsorption of carbaryl on active sites of the solid support and glass column where decomposition occurs. Lewis and Paris [108] indicated that by using a short column (0.3 m) and low column temperatures, thermal decomposition could be prevented. Using a gas chromatograph equipped with such a column containing 3% SE-30 on Gas-Chrom Q and a 63Ni electron capture detector, they were able to analyze for carbaryl in quantities as

small as 0.2-0.5 ng/injection volume. Again, both these methods were devised for residue analysis, and no information on their application to air sampling and analysis has been found. As a result of the lack of quantitative information on the sensitivity, accuracy, and precision of gas chromatographic methods as applied to analysis of air samples for carbaryl, such methods are not now recommended.

The p-nitrobenzenediazonium fluoroborate colorimetric method has been selected as the analytical method. This method has been used for many years by Union Carbide Corporation [13 (sec 11)] and others, [28,98-101] and has shown the required sensitivity for evaluation of personal exposures to carbaryl in accordance with the TWA environmental limit [97] discussed in Chapter V of this document. The principle of the method is that alcoholic potassium hydroxide is used to hydrolyze the carbaryl to 1-naphthol, which is determined colorimetrically after reaction with p-nitrobenzenediazonium fluoroborate. The presence of 1-naphthol in the air sample leads to erroneously high values for determination of the airborne concentrations of carbaryl. In addition, other substances such as phenols and aromatic amines that form derivatives with p-nitrobenzenediazonium fluoroborate or that absorb around 475 nm, will interfere if present in the air sample. Details of this method are presented in Appendix II.

When both 1-naphthol and carbaryl are in the air sample, gas chromatographic methods will probably allow separation of the two compounds, however, details for such methods are not worked out. When they are developed, our recommendations will be reconsidered. Since carbaryl is metabolized by humans to 1-naphthol, the recommended analytical method may be superior in detecting toxicity because of its lack of specificity.

Biologic Evaluation

Evaluation of the available literature cited in Chapter III would indicate that there are two possible means of determination of carbaryl exposure in the workplace environment. These are the measurement of 1-naphthol in the urine and determination of cholinesterase activity in the blood. The urinary concentration of 1-naphthol, one of the principal metabolites of carbaryl, which is excreted in the urine as the sulfate or glucuronide, [33,34] has been used as an indication of exposure to carbaryl in the workplace. [28] Comer et al [88] reported that the highest concentrations of urinary naphthol occurred usually after the daily exposure had ended. Because of the possible occurrence of carbaryl in food [109] and because 1-naphthol itself may be encountered during the manufacturing process, [15,16] many individuals exposed to carbaryl in the workplace might normally have small amounts of 1-napthol in the urine from sources other than airborne carbaryl. Although there is evidence that urinary 1-naphthol levels increase on exposure of humans to airborne carbaryl, [13 (sec 9,10),28,88] no definite quantitative relationship has been established between exposure to airborne carbaryl and total urinary 1-naphthol excretion. Therefore, measurement of urinary 1-naphthol as an indicator of carbaryl exposure is not now recommended.

Since the basis for the acute toxicity of anticholinesterase agents is the inhibition of the enzyme acetylcholinesterase, [7] the measurement of this enzyme activity would appear to be a useful biologic monitoring technique. The rapid regeneration of the carbamylated enzyme [5,110] probably makes the degree of inhibition by carbaryl at any given time elusive. The enzyme activity when analyzed in the sample tends to be higher

than was the actual case when the blood sample was obtained, and thus the degree of inhibition may be interpreted as less than actually occurred. [110] The California Department of Health [111] has recommended that, because of the reversibility of the complex of enzyme and carbamate, blood samples should be drawn and analyzed within 4 hours after exposure when carbamate poisoning is suspected.

Biologic monitoring of cholinesterase activity in blood, plasma, serum, or erythrocytes is not recommended as a means of assessing occupational exposure to carbaryl because (1) the rapid reversibility of carbaryl-induced cholinesterase inhibition makes cholinesterase determination impractical in occupational situations where blood samples cannot be analyzed immediately, and (2) occupational exposure to carbaryl results in a rapid onset of symptoms, self-termination from further exposure, and rapid recovery.

Although monitoring of cholinesterase activity is not specifically proposed as a requirement for carbaryl exposure, it can, properly performed, give useful information in exposures to carbaryl as well as to other cholinesterase-inhibiting compounds such as the organophosphate pesticides. For those who plan to carry out these measurements, it should be pointed out that individual variation due to disease states and genetic makeup may influence the results. A decrease in the activity of serum cholinesterase has been found in liver disease, [112] pregnancy, [113] and malignant neoplasms. [114] In patients suffering from pulmonary tuberculosis, plasma cholinesterase activity was also decreased. [115] Familial reduction in pseudocholinesterase levels was reported by Lehmann and Ryan [116] and by Kalow, [117] and later was found to be related to the

presence of an atypical gene. [118] Between 1 in 3,370 and 1 in 10,500 individuals tested in a healthy Canadian population was found to be homozygotic for this atypical gene [117] and thus could be expected to have a genetically determined deficiency in plasma cholinesterase. Erythrocyte cholinesterase activity was inhibited in certain pulmonary and extrapulmonary cancers [115] and in paroxysmal nocturnal hemoglobinuria. [119] Familial symptomatic reduction in erythrocyte cholinesterase activities also has been reported. [120] For those situations where measurements of cholinesterase activity is applicable, an assessment of cholinesterase methodology is presented below.

Measurement of cholinesterase (true or pseudo-) activity depends basically on two principles. The first involves measurement of the product formed as a result of hydrolysis of the substrate by the enzyme; this product can be estimated directly or indirectly by various techniques. The second principle involves measurement of the disappearance of the substrate, which also can be measured directly or indirectly by various techniques.

In early assays for cholinesterase, acetylcholine was usually used as a substrate and changes were measured as the ester was hydrolyzed. This change has been determined manometrically, [121,122] by changes in color of acid-base indicators, [123] and by pH measurement. [8]

Manometric methods for the analysis of cholinesterase are precise, but they are inherently tedious and time-consuming. These methods are based on the measurement of the amount of carbon dioxide released when acetic acid is allowed to react with bicarbonate ion. Both erythrocyte and plasma cholinesterase activities have been assayed manometrically. [7,124]

However, Winteringham and Disney [125] believed that since dilution of the enzyme was necessary to achieve sensitivity when using conventional manometric methods, these methods were inappropriate for measurements in cases of carbamate exposure. Such dilution decreased the percentage of enzyme bound to the carbamate substrate by enhancing the dissociation of the complex. [125]

A photometric method based on the change in color of an acid-base indicator has been adapted to measure cholinesterase activity in the presence of a large amount of serum by using a calibration based on the ratio of final to initial absorbance, [123] thereby eliminating the errors from the effects of dye binding with protein in the serum. This method is not applicable to hemolytic serum. Turbidity and extraneous colors interfere with the method. An automated method used by Winter [126] has not proved adequate for the assay of erythrocyte cholinesterase [127]; the automated micro determination of cholinesterase, as described by Levine et al, [127] involved a 1:4 dilution of plasma and serum and a 1:10 dilution of erythrocytes. Thus, these automated methods [126,127] have limitations resulting from dilution in determining carbaryl-induced cholinesterase inhibition. The principle of colorimetric or electrometric measurement of the acetic acid liberated from the hydrolysis of acetylcholine by cholinesterase has led to several useful field screening procedures for cholinesterase activity. Such field methods have poor precision (± 25%) and are adequate only for screening purposes. [128-131]

The electrometric method of Michel, [8] developed primarily to shorten the time required for analysis, was well suited to determinations of both erythrocyte and plasma cholinesterase activities. This method has

been used to measure erythrocyte and plasma cholinesterase activities in humans exposed to organophosphate cholinesterase inhibitors. [132] This method makes use of electrodes to measure changes in pH (expressed as delta pH/unit time) resulting from the production of acetic acid when cholinesterase hydrolyzes acetylcholine. However, when this method, [8] or modifications thereof, [132] are applied to the evaluation of carbamate exposures, problems are anticipated as a result of diluting the samples during the assay process.

More recently, Winteringham and Disney [133] devised a radiometric assay of acetylcholinesterase. In this method, a drop of 14C-labeled acetylcholine, used as the substrate, was mixed with the diluted blood sample on a slide. The enzymatic hydrolysis was stopped by adding a drop of acid inhibitor after a measured time period. The amount of unhydrolyzed 14C-labeled substrate was then measured with a Geiger-Muller tube after removal of volatile 14C-acetate by drying in hot air. This activity was then compared to that contained in a previously inactivated sample containing a measured amount of 14C-labeled substrate. By this method, the enzyme activity can be determined at low substrate concentrations and with minimal dilution.

Potter [134] modified the radiometric method to use both 14C- and tritium-labeled acetylcholine; the reaction product (labeled acetate) was quantified by liquid scintillation spectrometry after separation from the labeled acetylcholine by an extraction procedure. The advantage of the radiometric method is that the biologic fluids require no dilution, thereby preventing greater dissociation of the enzyme-carbamate complex. In a critical review of the measurement of cholinesterase activity, Wills [7]

suggested that the radiometric modification described by Potter [134] might be particularly appropriate for reversible inhibitors such as the carbamates. However, the WHO Expert Committee on Insecticides [29] did not recommend the radiometric method for general use. The expense, the necessity for complicated apparatus, and the difficulty in calculating the results were cited as disadvantages.

The basic principle of titrimetric techniques involves titration with standard alkali of the acid released during hydrolysis of the choline ester at a constant pH and use of either an indicator or a potentiometer. [110] According to Witter, [110] acetylcholine, acetyl-beta-methylcholine, or butyrylcholine can be used as the substrate in this method. Noting that the continually changing color in the reaction mixture was often difficult to match with the indicator standard, Witter [110] suggested that this difficulty was due to the color of the blood or the plasma, or to other factors such as the presence of activators or inhibitors. However, the author indicated that this difficulty was obviated if a potentiometer was used to measure the pH. Jensen-Holm et al [135] used an automatic titrator for measuring the cholinesterase activity in blood and other biologic material. The authors claimed that the method had the advantage of following the course of acid liberation (and therefore of cholinesterase activity) during the whole period of the determination. However, the method was subject to serious error due to nonspecific acid liberation in the organ homogenates before addition of substrate; but this error very rarely occurred in fresh blood. Another automatic titrimetric technique, [13 (sec 12)] in which cholinesterase activity of erythrocytes or plasma was determined in refrigerated equipment, was developed to retard the rapid

reversibility of the inhibition of cholinesterase activity by carbaryl. However, no data were reported on the samples analyzed.

Measurements of the production of acid by the addition of base to maintain a constant pH as hydrolysis of substrate occurs are referred to as pH-stat methods [7,136] and are automated versions of the method of Hall and Lucas. [137] Such methods have been used less frequently than the delta-pH methods in determining cholinesterase activity in plasma and erythrocytes. According to Crane et al, [138] they are slightly less precise than the delta-pH methods. Since these methods involve the addition of a base to neutralize the acid, they effectively increase the dilution of the sample and may therefore cause dissociation of the carbamate-enzyme complex. Therefore, pH-stat methods are of limited use in measuring carbaryl-induced cholinesterase inhibition.

Hestrin [50] reported on the stoichiometric reaction of acetylcholine with alkaline hydroxylamine to form acetohydroxamic acid which forms a red-purple complex with ferric chloride. Hestrin's work led to the development of other methods using the same principle for measuring serum cholinesterase activity by determining the amount of unhydrolyzed acetylcholine. [139,140] However, Hestrin's method [50] is not specific for acetylcholine; esters of other short-chain carboxylic acids, lactones, and anhydrides also react with hydroxylamine with resultant formation of hydroxamic acid.

Meyer and Wilbrandt [141] adapted a histochemical principle to the measurement of plasma and erythrocyte cholinesterase in blood based on the measurement of free SH-groups produced by the hydrolysis of esters of thiocholine by the active enzyme. Techniques using thiocholine esters have

been used to measure cholinesterase activity in plasma, serum, and erythrocytes, and have been adapted to automatic analytical equipment. [7] This measurement can be accomplished iodometrically, [141] colorimetrically, [142] or spectrophotometrically. [143] Ellman et al [143] were able to determine cholinesterase activity in blood, erythrocytes, and tissue homogenates, using the spectrophotometric method. The authors claimed several advantages for this method: it operates in the visible region of the spectrum, thus permitting spurious changes to be observed directly; it is extremely sensitive; and it requires no special handling of tissue homogenates. The colorimetric method of Gary and Routh [142] is a very rapid micro method, with only a 3-minute incubation period necessary, and has been used to determine serum and plasma cholinesterase activities. However, the application of these methods [141-143] to measuring carbaryl-induced cholinesterase inhibition is limited by the dilution required in the sample preparation.

Cranmer and Peoples [144] described a method for the determination of cholinesterase activity that involved the incubation of erythrocytes or plasma with 3,3'-dimethylbutyl acetate as a substrate. The reaction product, 3,3'-dimethylbutanol, was extracted into carbon disulfide and determined by gas-liquid chromatography. According to the authors, their method has been applied only to strong inhibitors of cholinesterase and not to reversible inhibitors. Therefore, its validity for assaying carbamate inhibition estimation has not been demonstrated.

Baum [145] described a method of measuring cholinesterase activity using a liquid membrane electrode having a high selectivity for acetylcholine. This method permitted a continuous determination of the

rate of change of substrate concentration in the presence of active enzyme. When the acetylcholine concentration fell below 0.1 μM, interference from sodium in the saline buffer solutions used became serious. No information was given on the applicability of this method for determining cholinesterase activity in whole blood or erythrocyte samples.