III. BIOLOGIC EFFECTS OF EXPOSURE

The carbamate insecticides, one of which is carbaryl, and the organophosphate insecticides as well, exert their insecticidal action by inhibiting cholinesterase enzymes. [1] This inhibition is the primary mechanism by which these insecticides cause toxicity in mammals. The cholinesterase enzymes hydrolyze acetylcholine and other choline esters; consequently, their inhibition leads to the accumulation of endogenous acetylcholine and other choline esters. Probably, most of the biologic effects of anticholinesterase agents, including carbaryl, are due to the inhibition of acetylcholinesterase which leads to the accumulation of endogenous acetylcholine, the principal choline ester that has demonstrated physiologic significance in humans.

Physiologically, acetylcholine is a chemical mediator of nerve impulses. [2 (pp 404-76)] It transmits nerve impulses to the heart and other parasympathetically innervated structures such as the muscles and glands of the gastrointestinal tract, including the salivary glands, the glands and muscles of the bronchial and urogenital systems, the sphincter muscle of the iris, and the ciliary muscle of the lens of the eye. A few postganglionic sympathetic fibers, such as those to the exocrine sweat glands, also release acetylcholine. In addition, acetylcholine has a neurotransmitter function at the termination of the preganglionic fibers on the neurons of the ganglia of both the sympathetic and parasympathetic parts of the autonomic nervous system and at the neuromuscular junction of

the peripheral nerves and skeletal muscles. Also, a role for acetylcholine as a transmitter in synapses in the CNS has been suggested. The inhibition of cholinesterase allows acetylcholine to accumulate at these sites and thereby leads to overstimulation of these innervated organs.

Based on the ability of horse serum to hydrolyze acetylcholine and butyrylcholine, Stedman et al, [3] in 1932, suggested the term "cholinesterase" to name the enzyme present in serum. Two principal types of enzymes that hydrolyze choline esters have been identified in humans as acetylcholinesterase, or true cholinesterase, and butyrylcholinesterase, variously referred to as pseudo-, serum, or plasma cholinesterase. [2 (pp 404-76)] Acetylcholinesterase catalyzes the breakdown of acetylcholine into acetic acid and choline. Acetylcholinesterase is found at nerve synaptic junctions, at the neuromuscular junction, in erythrocytes, and in other tissues. Plasma cholinesterase is present in various types of glial and satellite cells of the central and peripheral nervous systems, respectively, as well as in plasma, liver, and other organs. Its physiologic function is unknown; inhibition of plasma cholinesterase at most sites produces no apparent functional changes. Lehmann and Liddell [4] speculated that the function of plasma cholinesterase may be to hydrolyze those choline esters that inhibit acetylcholinesteras. These include propionylcholine and butyrylcholine, which can be formed in vitro by enzyme systems responsible for the synthesis of acetylcholine and by bacterial action in the gut. Occasionally, humans have atypical or deficient plasma cholinesterases, discovered through an investigation of abnormal responses to the muscle relaxant succinylcholine, which appear to be genetically controlled variants having differing abilities to hydrolyze

acetylcholine and related compounds. [2 (p 585)]

O'Brien [5] discussed several theories on the mechanism of cholinesterase inhibition by carbamates including carbaryl. One theory which the author proposed after a critical review of the literature on carbamates is briefly described. Carbamates react with cholinesterase in a way similar to the reaction between organophosphates and cholinesterase. An immediate and direct reaction takes place between the carbamate and cholinesterase, resulting in the formation of a reversible enzyme-inhibitor complex. Second, in the case of carbaryl, carbamylation of the enzyme occurs with the release of 1-naphthol. Third, hydrolysis (decarbamylation) of the carbamylated enzyme occurs with regeneration of the original enzyme. The rate at which this step proceeds is a major determinant of the rate at which the inactive enzyme is regenerated or restored to function. With the organophosphate insecticides, the third stage is extremely slow, whereas for the carbamate insecticides, eg, carbaryl, it is relatively rapid with a calculated half-life of 40 minutes for decarbamylation of the enzyme. In addition, if at any stage of the reaction the concentration of the carbamate is reduced, eg, by dilution, the carbamylated enzyme recovers activity by dissociation of the enzyme-inhibitor complex and by hydrolysis of the carbamylated enzyme. Thus, the carbamylated enzyme can be readily and rapidly regenerated and restored to function following inhibition. Therefore, one may assume that inhibition of circulating cholinesterase may not be as readily apparent following exposure to carbaryl as with other cholinesterase inhibitors. This assumption is also supported by the discovery of the ability of a component of human serum albumin to metabolize carbaryl to 1-naphthol, [6] and by the fact that most methods

for determination of cholinesterase activity require dilution of the enzyme-substrate complex, thus accelerating the dissociation. [7] The method most frequently used for the measurement of cholinesterase activity, except in carbamate inhibition, is that of Michel. [8] Using 800 "healthy" blood donors, Rider et al [9] established normal limits of 0.56-0.95 and 0.38-1.39 delta pH/hour for erythrocyte and plasma activities, respectively, by the electrometric method. [8] The donors were men and women between 18 and 60 years of age. Carbaryl differs further from many anticholinesterase agents in that its metabolic changes result in deactivation, [10] while some of the organophosphate insecticides (the thiophosphates) require metabolic activation to produce an effect. [5]

Extent of Exposure

Carbaryl ($C_{12}H_{11}O_2N$) is known chemically as 1-naphthyl N-methyl-carbamate. Table XIII-1 presents the more important physical properties of carbaryl. [11,12,13 (sec 16), HH Moorefield, written communication, February 1976] It is sold in the United States under the trade name Sevin and Table XIII-2 [14] lists its trade names and synonyms.

Carbaryl can be produced by reacting 1-naphthol and methyl isocyanate. [15] Also, phosgene and 1-naphthol can be combined to produce naphthyl chloroformate which reacts with methylamine to produce carbaryl. As the exact sequence of the domestic carbaryl-manufacturing process is not known, it has been suggested that the first production method is probably used. [16]

Total carbaryl production in the United States was estimated to be about 50 million pounds for each of the years 1967 and 1970, [16,17]

45 million pounds in 1971, [18] and 53 million pounds in 1972. [19] Of the total produced in 1972, approximately 28 million pounds were exported. About 25 million pounds were used in the United States, 19 million for agricultural purposes, 3.5 million in homes and gardens, 1.5 million by government, and 1 million for industrial and commercial purposes. Slightly over half the carbaryl used nationwide in 1972 was used in the southeast and the north central states (about 8 million and 5 million pounds, respectively). [19] An estimated 40% of the carbaryl used throughout the world is applied in the production of cotton. [20]

The product of the carbaryl-manufacturing process is a 98% concentrate. [21] Carbaryl is formulated as wettable powders, pellets, granules, dusts, fertilizer mixtures, and liquids. [18]

The people most likely to be occupationally exposed to carbaryl are those engaged in the development, manufacture, and distribution of insecticides, as well as agricultural crop workers, farmers, plant nursery workers, spray pilots, and others engaged in spraying and dusting operations, eg, soil fumigators and exterminators. [22] NIOSH estimates that 100,000 US workers are potentially exposed to carbaryl.

Historical Reports

The cholinesterase-inhibitor physostigmine, an alkaloid extract of the calabar bean, has been used since the mid-19th century for ophthalmologic treatment and diagnostic purposes. [23] Its clinical use predates an understanding of cholinesterase enzyme inhibition.

In 1925, Stedman and Barger [24] elucidated the structure of physostigmine. A year later, Stedman [25] reported on the pharmacologic

action of substituted phenyl carbamates in mammals. He had synthesized a series of compounds and screened them for miotic activity by applying them directly to the eyes of cats.

Research and development on heterocyclic carbamates, initiated in 1947, led to several Swiss patents on dimethylcarbamates having insecticidal activity. [26] Lambrech synthesized carbaryl (Sevin) in the United States in 1953; and in 1956, after preliminary field tests, Sevin was released for testing in agricultural experiment stations. [20] In 1957, Haynes et al [26] described the insecticidal properties and some of the physicochemical characteristics of carbaryl.

Union Carbide Corporation was issued US Patent No. 2,903,478 for carbaryl in 1959, [18] the year after commercial production and sales on an experimental basis were begun. [16,20]

In 1961, Carpenter et al [27] described a series of biologic and toxicologic investigations of carbaryl conducted on experimental animals. In 1962, Best and Murray [28] were the first to report the results of investigations on occupational exposures to carbaryl. These investigations [27,28] are discussed further in this chapter.

Best and Murray [28] described the case of a 19-month-old infant who had miosis, excessive salivation, and incoordination after ingesting an unknown amount of carbaryl. The child responded to gastric lavage and atropine therapy and apparently recovered completely in 12 hours. The authors [28] received a report of this incident in a personal communication, and it probably is the first reported case of symptomatic carbaryl poisoning.

In 1963, Hayes [21] reported that, within 20 minutes after ingesting a 250-mg "carefully measured oral dose" (about 2.8 mg/kg) of carbaryl, a man experienced epigastric pain and sweating. Atropine sulfate was administered, and recovery was complete within 2 hours. Hayes gave no further details on the incident. A later publication [29] indicated that the incident described in 1963 was, in fact, a purposeful ingestion by a scientist.

Effects on Humans

Feldmann and Maibach [30] studied the percutaneous absorption of carbaryl by applying 14C-labeled carbaryl (4 μg/sq cm) dissolved in acetone to the forearm skin of six adult male volunteers. The acetone volatilized in seconds; the skin was not washed for 24 hours. Eight urine specimens were collected from each subject over 5 days as follows: four during the first day, three collections at 4-hour intervals and the fourth 12 hours later; and once daily (total 24-hour specimens) on each of the next 4 days. The amounts of 14C radioactivity in the urine were determined by liquid scintillation counting and were compared with the amount of 14C radioactivity recovered in a similar study involving a single intravenous (iv) injection of one microcurie of carbaryl. After correction with the factor of 7.4% for incomplete urinary excretion obtained in the iv study, the results were expressed in percentages of the dermally applied dose. The results indicated that 73.9% of the 14C-carbaryl applied to the skin was excreted in the urine within 5 days. The absorption of carbaryl was probably increased by the use of acetone in this study.

In a similar study of 14C-carbaryl (4 μg/sq cm) dissolved in acetone,

Maibach et al [31] showed that other parts of human skin were similar to that of the forearm in absorption of carbaryl. Approximately 70% of the 14C-carbaryl was excreted within 5 days after application to the skin over the angle of the jaw. Other pesticides were also studied. Parathion absorption by the skin of the scrotum, axilla, and forehead, for example, was considerably greater than that from the forearm skin.

Wills et al [32] collected urine from two male volunteers who had been administered carbaryl in gelatin capsules at a dose of 2 mg/kg. Total urine samples were collected at 4-hour intervals for the first 16 hours, followed by one 8-hour collection sample. Thereafter, for the next 3 days, total urine was collected and pooled at 24-hour intervals. Urine samples from control subjects had been obtained for the 24-hour period before carbaryl administration. Using these samples, Knaak et al [33] determined the metabolites of carbaryl in the urine of the two volunteers. Portions of all urine samples were subjected to ion-exchange chromatography, and fractions were analyzed by spectrophotofluorometry. Also, composite 4-day samples, one from each subject, were pooled for colorimetric analysis. The metabolites in the 24-hour urine samples, separated by chromatography, were 1-naphthyl glucuronide, unidentified neutrals, 4-(methylcarbamoyloxy)-1-naphthyl glucuronide, and 1-naphthyl sulfate. In addition, another metabolite, 1-naphthyl methylimidocarbonate O-glucuronide, was identified by fluorometry. Moreover, colorimetric analysis revealed an average concentration of 1-naphthol in the urine of 0.81 mg/100 ml. From this, the authors estimated that 37.8% of the orally administered carbaryl would be excreted within 4 days. Of the total determined, fluorometric analysis indicated that only 26-27% was excreted as metabolites during the first 24

hours. These metabolites were identified and quantitated. They were: 1-naphthyl glucuronide 12.9%, 1-naphthyl sulfate 8.5%, and 4-(methylcarbamoyloxy)-1-naphthyl glucuronide 5.1%. Fluorometric analysis revealed small amounts of metabolites in the urine on the second day but none were detected on days 3 and 4. Knaak et al [33] did not explore the possibility that either carbaryl or some of its metabolites may have been excreted in the feces, either directly or indirectly through the enterohepatic circulation, or by other routes.

Using similar techniques in another study, Knaak et al [34] analyzed 24-hour urine specimens from men exposed to carbaryl dust during a packaging operation in a factory (the number of men, exposure periods, and other conditions were unspecified). Twenty-four-hour urine specimens obtained from the same men 72 hours after their last exposure to carbaryl served as controls. The urinary metabolites separated by chromatography were 1-naphthyl glucuronide, 1-naphthyl sulfate, and unidentified neutral metabolites. Concentrations of the glucuronide and sulfate in the urine were estimated to be 25 and 5 μg/ml, respectively.

Vandekar [35] published the results of a study conducted in 1963 by the WHO Insecticide Testing Unit in three villages of southern Nigeria. The investigators used a water-dispersible carbaryl powder with 85% active ingredient to prepare a spray that contained 5% active ingredient which they expected would deposit 2 g of active ingredient on each square meter of surface. They studied the effects on 10 men who applied the spray as a residual insecticide in 16 houses over a single 6-hour period and on 95 villagers who resided in these houses. The sprayers wore overalls, broad-brimmed oilskin hats, and rubber boots during the entire 6 hours of

spraying, but wore masks for the first hour only. Clinical observations of all 105 individuals involved were recorded throughout the investigation. Plasma cholinesterase activities as well as urinary metabolites were determined before and after spraying. Except for a distinct rash on a single sprayer whose back was splashed while he was filling an applicator with the carbaryl spray, no adverse effects were seen in either the sprayers or the villagers. Plasma cholinesterase activity was determined electrometrically by a micromodification [36] of the method of Michel from blood samples taken by fingerprick and stored at 4 C until analyzed, usually on the same day. This method, like most others, probably underestimates the amount of inhibition of cholinesterase activity caused by carbaryl (see Biologic Evaluation in Chapter IV). A slight reduction in plasma cholinesterase activity was reported [35] for all 10 sprayers the day after the carbaryl application; however, when measured again 5 days after spraying, the enzyme activity was found to be within the preexposure range. A slight but statistically significant reduction in plasma cholinesterase activity was found in the 63 exposed villagers measured 1 week after the spraying. The mean reduction was 8%, ranging from somewhat greater for residents 7- to 14-years-old (10.1%) and those over 30 years of age (10.8%), to less for residents 1- to 6-years-old (5.9%) and for those 15- to 30-years-old (5.3%). The metabolites of carbaryl were estimated [35] according to the colorimetric method of Dawson et al [37] in urine samples usually collected between 8 AM and 10 AM. Excretory levels of naphthol derivatives in the urine of the sprayers were unchanged on the first and second days or thereafter, except for a slight increase on the sixth day after exposure to carbaryl. [35] The urinary excretory levels of

naphthol derivatives for 38 villagers before and for 32 villagers a week after the spraying, however, were significantly different; before exposure, the mean was 30.5 μg/ml, and 50.3 μg/ml afterwards.

In a comprehensive Union Carbide Corporation report submitted to NIOSH, Williams [13 (sec 9,10)] described an inhalation-absorption study of two employees. Employee A, an observer, was located near employee B who was emptying containers of carbaryl in a recovery operation. Employee A had complete body protection, except for the face, but no respiratory protection. Employee B, who made no special effort to avoid body contact, worked with and without gloves, but wore coveralls and an air-supplied respirator. Although observation extended intermittently over 24 days, the carbaryl airborne concentrations were similar on only 2 days. On the first such day, the airborne environmental carbaryl concentrations were 50.9 and 49.3 mg/cu m for employees A and B, respectively. The initial urinary naphthol concentration of employee A obtained before he began work that day was undetectable. After exposure to carbaryl, it rose to a maximum of 3,619 μg/100 ml. For employee B, the initial urinary naphthol concentration of 1,939 μg/100 ml rose to 8,975 μg/100 ml. Both maximum concentrations were from urine samples collected at 6:30 PM, after exposure had ceased. On the second day, similar environmental airborne carbaryl concentrations, 45.2 and 40.6 mg/cu m, were reported for employees A and B, respectively. The initial (second-day) urinary naphthol value for employee A was again undetectable, and the maximum, 2,430 μg/100 ml, was in the sample taken at 5:30 PM. On day 2, corresponding urinary naphthol concentrations for employee B were 1,803 μg/100 ml initially and 2,340 μg/100 ml at 9:30 PM. No noticeable effects on either employee were

observed or expressed; there were no pinpoint pupils, thought to be an early sign of excessive exposure to carbaryl. Methods used for air sampling and analysis were not identified, nor was the method for determining urinary 1-naphthol described.

Long [38] recounted an incident in which a worker, wearing a mask and goggles (skin protection not described) while loading an airplane with a mixture of carbaryl and sulfur, indicated he was weak and dizzy and could not get his breath. He experienced the same symptoms 2 days later, at which time chemical toxemia was the diagnosis. The attending physician noted that, according to the employer, four other employees had been sick or had had similar attacks. Long presented no further details.

Yakim [39] reported in limited detail investigations of the biologic effects of carbaryl in agricultural workers in the USSR. Whole blood cholinesterase activity was determined in men after 4- to 6-hour exposures to airborne concentrations of carbaryl for 3-4 days. The method used to determine blood cholinesterase activity was not specified. Cholinesterase activities decreased 11-22% in men exposed at a mean airborne carbaryl concentration of 2 mg/cu m while working at a loading site. Tractor drivers who disseminated carbaryl and were exposed at an average concentration of 2 mg/cu m of air had a fall of 20-24% in blood cholinesterase activity. Signalers exposed at 4 mg/cu m experienced a 13-30% decrease in cholinesterase activity. Yakim [39] indicated that the blood picture and results of certain tests of physiologic functions (pulse rate, body temperature, Aschner's reflex (oculocardiac), dermographism, and what was described as an orthoclinostatic test) suggested no change in these workers before and after work. Pilots engaged in aerial application

were exposed in the cabin at a mean airborne carbaryl concentration of 7 mg/cu m, but Yakim [39] reported no data on the changes, if any, in their cholinesterase activity or in the blood picture or physiologic functions.

Farago [40] presented the details of a suicide attributed to carbaryl ingestion. The victim, a 39-year-old man described as inebriated, ingested about 0.5 liter of a solution of Sevin 80 (possibly 400 g of carbaryl). He was hospitalized 1.5 hours later, and his stomach was lavaged and circulation-promoting agents were administered. As his condition deteriorated, the patient complained of disturbed vision, and lung edema developed. He received atropine every half hour. One and a half hours after entering the hospital, he also received 250 mg of pralidoxime (PAM). Hayes [21] pointed out that the use of PAM is inappropriate in the treatment of carbaryl intoxication (see Appendix III for medical management). Following the administration of PAM, his condition deteriorated even more rapidly with attendant signs of advancing pulmonary edema. [40] The man died 6 hours after ingesting the carbaryl. Autopsy revealed what was described as dark, livid red spots on the body surface; swollen, edematous brain tissue; severe pulmonary edema; and blood-congested viscera. Farago [40] considered the degree of kidney hyperemia present especially striking. Analysis of the organs showed the following carbaryl concentrations (in mg%): stomach flushing water, 244.6; stomach and contents, 14.8; intestine and contents, 17.6; blood, 1.4; liver, 2.9; kidneys, 2.5; and urine, 3.1. At the time of death, carbaryl had not been completely absorbed from the gastrointestinal tract; that which had been absorbed apparently accumulated in the liver and kidneys. The cholinesterase activity in the blood, determined by an electrometric

method, was strongly inhibited. Thin-layer chromatography revealed one or two (not clear from the paper) metabolites of carbaryl in the stomach, three metabolites in the intestines, four in the liver and kidneys, and five in the urine. None of the metabolites were identified. The presence of a metabolite in the stomach suggests that some metabolism may also occur at that site in human beings.

Of the 25 cases of poisoning from organophosphate and carbamate compounds described by Lopez, [41] only two were attributable to carbaryl alone. The first was a 19-year-old man who ingested watermelon sprayed with 80% carbaryl, and the second was a 33-year-old man who inadvertently drank several milliliters of a solution of 80% carbaryl. Lopez [41] classified the degree of intoxication in both men as light. In addition to nausea, both subjects experienced signs and symptoms including vomiting, hyperreflexia, pallor, intestinal colic, and nasal discharge in the first victim; and salivation, headache, lacrimation, and tremors in the second. The 19-year-old and the 33-year-old patients were treated with 20 mg and 5 mg of deoxycorticosterone and recovered completely in 18 hours and 1.5 hours, respectively. The author hypothesized that either corticoids liberate cholinesterase bound to serum proteins or that further induction of this enzyme had been initiated by deoxycorticosterone.

In a Union Carbide Corporation medical department report dated June 19, 1962 and provided to NIOSH, Sexton [13 (sec 7)] reported an incident at a carbaryl-shipping facility where storage bins had become plugged, necessitating hand-removal of carbaryl dust from hoppers leading to the bins. Fourteen employees were involved, and all of the men were supposedly wearing dust respirators all the time while working. Between early and

late afternoon, 7 of the 14 men reported to the medical department complaining of symptoms they attributed to inhalation of carbaryl dust. It was interpreted that the felt cartridge of the respirators had become clogged with dust, so that not only dust but also the vaporized carbaryl may have passed through the filter cartridge, resulting in exposure. The workers had complaints of nausea, dizziness, or both. In addition, one man complained of headache and another of being "overheated" and perspiring. The seven men had worked 8-16 hours on the day they became ill. The episode occurred on a Saturday afternoon, and company physicians were not notified until the following Monday morning. At that time, all 14 employees were seen at the medical department, where urine and blood specimens were collected. By then, none of the 14 men had any complaints. Blood cholinesterase activities were measured by an electrometric method which probably underestimated the degree of cholinesterase inhibition. Urine was analyzed for conjugated naphthol. The mean conjugated naphthol content of the urine of the seven men who had complained of illness was 1,417 μg (range 200-3,100)/100 cc. The mean conjugated naphthol content of the urine of the seven asymptomatic workers was 2,443 μg (range 1,000-4,200)/100 cc. Mean cholinesterase activities for the ill workers were 1.097 (erythrocyte) and 3.183 (plasma) delta pH units, and for the well employees, 1.258 (erythrocyte) and 3.368 (plasma) delta pH units. No environmental data were reported. Medical personnel concluded that the effects were due to inhalation of carbaryl, related perhaps to respirator malfunction. However, the intoxications reported could have resulted from other than lung absorption.

In a 1970 memorandum by Dernehl [13 (sec 13)] submitted to NIOSH by Union Carbide Corporation, the sequence of events in Union Carbide workers overexposed to carbaryl during its manufacture, formulation, and use was summarized as follows: headache and nausea after 3-4 hours of continuous overexposure to airborne carbaryl dust at unstated concentrations; vomiting, possibly accompanied by mild abdominal cramps, about 30 minutes after the onset of headache and nausea; dimness of vision; and termination of exposure because of the vomiting. Dernehl [13 (sec 13)] added that by the time affected employees were provided medical attention, usually 30-45 minutes after becoming ill, symptoms already had begun to disappear, and only in rare instances did a physician consider atropine sulfate administration necessary. He also pointed out that it was rarely possible to quantitatively determine hazardous exposure levels, but that the employees' verbal descriptions suggested heavy concentrations well above those normally encountered.

Wills et al, [32] in a preliminary study, administered carbaryl in gelatin capsules to male volunteers 25-57 years of age (mean 36). Single oral doses of approximately 0.5, 1.0, and 2.0 mg/kg of carbaryl were given to three pairs of men, who were then observed for signs of intoxication. Blood plasma and whole blood cholinesterase activities were monitored by a pH stat method, and the subjects were questioned about symptomatic effects. Neither objective nor subjective changes were noted in the preliminary study. Wills et al [32] also administered carbaryl to another series of subjects. Two groups of five men each received either placebo capsules or capsules containing 0.06 mg/kg of carbaryl daily for 6 weeks. Two weeks later, two new groups of six men each started ingesting, again for six

weeks, either placebo capsules or doses of carbaryl analyzed at 0.12 mg/kg by one method and by another at 0.13 mg/kg. Blood, urine, and fecal specimens were collected initially and weekly from all subjects throughout each of the 6-week study phases. The authors specifically noted that sulfobromophthalein (BSP) retention in the plasma before and after carbaryl administration did not differ, that cholinesterase activities were not significantly altered, and that no significant effects attributable to carbaryl were present in the hemograms, in the blood chemistry, or in urine or fecal examinations. Blood samples were examined for hematocrit, hemoglobin, erythrocyte count, total and differential leukocyte counts, blood urea nitrogen (BUN), glucose, plasma and whole blood cholinesterase activities, cholesterol, prothrombin time, serum glutamic oxaloacetic transaminase (SGOT), sodium, and potassium. Urinary specimens were examined for turbidity, pH, protein, glucose, erythrocytes, squamous epithelial cells, crystals, and for the ratio of amino acid nitrogen to creatinine. Stool specimens were examined for occult blood. The authors indicated that final EEG recordings for both the treated and the untreated groups were somewhat more synchronized than were the initial ones but showed none of the spiking reportedly found in the EEG of subjects exposed to other (organophosphate) cholinesterase inhibitors. Subjects receiving carbaryl at the higher dose expressed several complaints not reported by the corresponding placebo group. Because most of these occurred the day after ingestion of carbaryl had ended, they were thought to be manifestations of withdrawal. Of the remaining complaints, the authors considered the two instances of difficulty in sleeping and of abdominal cramps to be symptoms of cholinesterase inhibition. Only one subject

reported the third complaint, an eye reaction (pupillary dilation), on one day; since this response usually is not caused by acetylcholinesterase inhibition, the authors attached no particular significance to it.

The only deviations from control levels in all the determinations reported were in the amino acid nitrogen to creatinine ratios, considered an estimate of the reabsorptive capacity of the proximal convoluted tubules of the kidney. [32] Wills et al [32] illustrated in charts the averages and ranges of these ratios for the men on both doses of carbaryl and for their controls, but did not state the actual numerical results. Ratios of the low-dose group (0.06 mg/kg) and their controls were determined initially and at weeks 1, 2, 4, and 6 of the study. Those ratios (low dose) were consistently below the controls except at week 6, when they were equal. Determinations were similarly recorded for the high-dose (0.12 or 0.13 mg/kg) group and their controls. Averages for six control and for six experimental subjects (five at the final determination) were compared. The ratios of the experimental group fell below those of the controls at the end of the first week of carbaryl ingestion, spiked upward at week 2, and fell to a plateau (at about midpoint of the second week's increase), where they remained until the fifth week. The next determinations on four of the subjects, 15 weeks after the last dose, showed that the amino acid to creatinine ratios had returned to the control level. The authors concluded that men who took carbaryl by mouth in daily doses of 0.06 or 0.12 mg/kg for 6 weeks suffered no subjective or objective changes clearly attributable to carbaryl other than a slight, reversible decrease in the ability of the proximal convoluted tubule of the kidney to reabsorb amino acids in the group on the higher dose.

Back, [20] in a 1965 review of product development experiences with carbaryl, referred to "about 50 cases" of intoxication, none fatal, and "less than a dozen" instances of cholinesterase inhibition, apparently due to the carbamate. He noted that, in those instances when exposure had occurred among process workers, formulators, or applicators, the onset of illness consistently resulted in cessation of work and thus of further exposure. The author gave no other details.

Epidemiologic Studies

Best and Murray [28] reported observations made during a 19-month period in a carbaryl-manufacturing plant. Involved in the survey were 59 employees, some of whom were exposed to carbaryl for less than 1 week. A total of 81 samples of airborne particulates (or air samples) were collected on Millipore filters (AA white grid, 47-mm Millipore filter paper). The concentration of carbaryl was determined colorimetrically using the reaction with p-nitrobenzenediazonium fluoroborate. The airborne concentrations of carbaryl under normal and abnormal conditions in the production area, air separator house, stacking and shipping area, and bagging areas are shown in Table III-1. In addition to the work areas listed above, the airborne carbaryl concentrations to which the building services employees were exposed, while not measured, were estimated to be relatively high, because these employees were exposed to dusty jobs in all plant areas. Employees were required to wear respirators only while cleaning the air separator house and at times when high concentrations were anticipated in the bagging areas. The range of concentrations of the 81

TABLE III-1

CARBARYL CONCENTRATIONS IN A MANUFACTURING PLANT

Location	No. of Samples	Concentration in mg/cu m	
		Range	Mean
Production area	49	0.03 - 0.73	0.23
Air separator house	2	19 - 34	31
Stacking and shipping area	6	0.05 - 1.52	0.64
Bagging area (normal)	18	0.20 - 1.60	0.75
Bagging area (abnormal)	6	19 - 40	29

Adapted from Best and Murray [28]

air samples was 0.03-40 mg/cu m. Only eight of the samples equaled or exceeded an airborne concentration of carbaryl of 19 mg/cu m. Of these, two were from the air separator house (29 and 34 mg/cu m), and six were recorded in the bagging area under abnormal conditions, such as when flow system connections or bags broke, or when bags were difficult to fill (range 19-40 mg/cu m). The other 73 samples had carbaryl concentrations equal to or less than 1.6 mg/cu m.

During the first 4.5 months of the Best and Murray [28] study, urine specimens for 1-naphthol determination were taken early in the morning before work on the day after blood samples were drawn for cholinesterase determination. Cholinesterase activity measured by an insensitive method was reported to be only slightly lowered, but since 1-naphthol excretion was significantly high in 10 of 63 urine specimens from employees likely to

have been most heavily exposed, it seems likely that cholinesterase activity might have been affected. For the next 3.5 months, 47 urine samples taken in the evening on days blood was drawn showed an average of 35.4 μg/100 ml which was approximately 9 μg/100 ml more 1-naphthol than that in samples taken the next morning. During the same 3.5-month period, most of the cholinesterase activities fell again from 96-100% of the normal to 70-95% of normal range, measured by an insensitive method. Over the next 3 months, when carbaryl production, bagging, and shipping ceased entirely, the 1-naphthol urinary levels of the above employees decreased to an average of 10.8 μg/100 ml. With resumption of carbaryl-manufacturing operations and of employee blood and urine sampling for the following 8 months, 23% of 138 exposed employee blood samples, but no control blood samples, had some reduction in cholinesterase activity; in this case, the quantitative method of Fleisher and Pope [42] was used but this probably underestimates carbaryl-induced cholinesterase inhibition. In the relatively high exposure groups, blood samples from 5 of 35 workers in the bagging areas, 13 of 67 workers in building services, 6 of 89 workers in production, and 6 of 78 workers in shipping had enzyme activities measured by the method of Fleisher and Pope [42] that were somewhat lower than those from the control group of unexposed workers. With these exceptions, blood cholinesterase activities in the other exposed workers did not vary significantly from those of the controls. The authors did not specify either the basis for selection of the control group or the time when blood samples were taken. The 1-naphthol findings in the same group were much more impressive; 41% (of 138 specimens) had average values of more than 1,000 μg/100 ml, 2.5 times the highest control levels (150-400 μg/100 ml).

In another study by Best and Murray, [28] the 1-naphthol urinary output determined during one workweek was more than doubled in one of two carbaryl baggers. Five other employees regularly assigned to various duties were monitored by 1-naphthol urine determinations twice daily for a single Monday-through-Friday workweek. The 1-naphthol levels in the urine were approximately 150% of their own control levels in two workers and approximately 223, 277, and 285% of their control levels in the three other workers at the end of the workweek. Although all of these employees had been similarly exposed to carbaryl dust in the previous week, they began on Monday of the test week usually with low values of urine 1-naphthol. The 1-naphthol urinary levels were highest on Wednesday to Friday. In the study of two baggers, one, who had been on vacation the previous week, began on Monday with urinary levels in the normal range and never reached the level of the other bagger.

In summarizing their results, Best and Murray [28] stated that relatively large amounts of 1-naphthol were excreted in the urine by employees exposed at air concentrations of carbaryl ranging from 0.23 to 31 mg/cu m but at times reported to be 0.03-40 mg/cu m. Blood cholinesterase activities in exposed employees were either within the normal range or were only slightly inhibited, but, in view of the enzyme assay methods used, these data are of doubtful significance. At no time did any of the employees studied have clinical or subjective evidence of increased acetylcholine activity.

Animal Toxicity

(a) Toxicity Studies

(1) Acute toxicity (other than inhalation) and interactions

Several investigators [27,43,44] have studied the acute oral toxicity of carbaryl in laboratory animals. Carpenter et al [27] calculated the LD50 of carbaryl based on mortality occurring within 14 days after administration by oral gavage of single, graded doses of the test material to groups of five rats weighing 90-120 g. The carbaryl was administered at a concentration of 50 mg/ml in 0.25% agar. The LD50's were 510 mg/kg in rats of unspecified sex and 610 mg/kg in females. Other authors, [43,44] using different strains of rats or different suspending agents, reported slightly different LD50 values. For example, Gaines [43] suspended technical grade carbaryl in peanut oil for oral administration to rats and calculated the LD50 values of carbaryl as 850 and 500 mg/kg for male and female rats, respectively. Gaines [43] also reported that the lowest oral dose which killed a male and a female rat was 600 and 100 mg/kg, respectively. These results suggested that the female rat was more sensitive to carbaryl than was the male. Coulston and Serrone [44] determined the oral LD50 in rats (sex not stated) to be approximately 600 mg/kg. The same authors reported an oral LD50 for mice of 650 mg/kg and an estimated LD50 for dogs and monkeys at less than 500 and greater than 1,000 mg/kg, respectively.

In a series of studies designed to assess the extent of cholinesterase inhibition in animals after administration of carbaryl, Carpenter et al [27] injected carbaryl iv as an 8% solution in 95% ethyl alcohol at doses of 10 and 15 mg/kg of body weight to two groups of three

dogs. Blood samples were taken before, and at intervals of 0.5, 1, 2, 5, and 23 hours after, carbaryl administration. Erythrocyte and plasma cholinesterase activities were determined by an electrometric method which, like many other methods, probably underestimates the degree of inhibition. Ethyl alcohol administered iv at volumes approximating the amount of ethyl alcohol given in the 8% carbaryl solution did not affect either plasma or erythrocyte cholinesterase activity. Recognizing the difficulties in evaluating the rapid reversibility of carbaryl-inhibited cholinesterase activity, the investigators chose to measure the extent of inhibition after equilibrium between substrate and enzyme was attained in the incubation phase of the analysis. They conceded that the values thus attained were not an exact measure of cholinesterase activity, but they believed that the values did represent the relative picture at various time intervals after administration. Results of these determinations revealed no significant changes in either erythrocyte or plasma cholinesterase activities after single iv carbaryl doses of 10 and 15 mg/kg.

Carpenter et al [27] also administered carbaryl in single oral doses by gelatin capsule to six bitches weighing about 8 kg each. One dog received carbaryl at 500 mg/kg, four dogs at 375 mg/kg each, and one dog at 250 mg/kg. The one dog given 250 mg/kg remained normal; the five dogs at the two highest doses showed signs of overstimulation of the parasympathetic nervous system. The signs the authors reported were as follows: the dogs were quiet for 15-30 minutes; then salivation and respiratory rate increased; lacrimation, urination, defecation, and intermittent muscular twitching occurred for 30-90 minutes; and muscle tremors increased (one dog had a minor convulsion). All five dogs had

constriction of pupils, profuse salivation, poor coordination, diarrhea, further increases in respiratory rate, and loss of bladder control after 2.5 hours. Three hours after administration, the animals vomited mucus and thick saliva and had violent intestinal movements, weakness, and considerable muscular spasm. After 5.5 hours, all five animals became quieter, but lacrimation, salivation, slight pupillary constriction, poor coordination, intermittent muscular twitching, and occasional vomiting of mucus persisted. After 7 hours, the pupils were almost normal, salivation decreased, and coordination improved. The authors noted no adverse effects on the following day, except that the dog treated with 500 mg/kg of carbaryl was extremely weak for 24 hours and did not eat for five days. Cholinesterase activities were not determined in the animal receiving 500 mg/kg. Plasma cholinesterase inhibition was found to be "insignificant" in the four dogs given 375 mg/kg, but some erythrocyte cholinesterase inhibition was verified in three of them, the maximum effect occurring within 2-3 hours after carbaryl administration. The maximum inhibition of erythrocyte cholinesterase activity during the first 3 hours for the three dogs was 24-33%. After 7 and 24 hours, these values varied from 12-30% and from 0-14%, respectively.

Carpenter et al [27] studied the effectiveness of atropine sulfate (an agent that blocks the cholinergic receptors, particularly at the parasympathetic neuroeffector junction, to prevent the action of acetylcholine) in combination with pyridine-2-aldoxime methiodide (PAM) (an agent that reactivates the phosphorylated cholinesterase enzymes after poisoning with organophosphate inhibitors) in blocking cholinergic effects produced by a single high dose of carbaryl in the dog. A single dog

weighing 8 kg, which had received carbaryl at a dose of 375 mg/kg in a capsule, was treated with 40 mg of PAM and 10 mg of atropine sulfate after signs of poisoning appeared. This combination failed to control the effects of carbaryl, but subsequent doses of 5 and 10 mg of atropine sulfate without the PAM proved effective. The same investigators administered a lethal oral dose (800 mg/kg) of carbaryl followed by PAM at a dose of 20 mg/kg iv to five female rats with no reduction of the mortality rate. A similar dose of atropine sulfate prevented death in a similarly treated group of five female rats. Similar results were obtained in rabbits. All these studies appear to provide reliable evidence that the administration of large doses of atropine sulfate, but not of PAM, can be effective in treating carbaryl intoxication. The adverse effect of oxime cholinesterase reactivators in animals poisoned with carbaryl was confirmed by Natoff and Reiff [45] in rats and by Akamatsu and Kohgo [46] in mice. Sanderson [47] found oxime treatment ineffective in carbaryl poisoning in rats.

In order to study the interactions between carbaryl and other pesticides, Carpenter et al [27] administered single oral doses of carbaryl in combination with each of 24 other compounds by gavage to rats weighing 90-120 g. The predicted LD50 for each pair of pesticides was calculated from the individual LD50 and proportion of each compound present in the administered dose. The ratio of the predicted toxicity over the observed toxicity was calculated. The authors considered a ratio of two or more indicative of a greater than additive or potentiating effect. They concluded from their data that there was no evidence of carbaryl-caused potentiation or antagonism. However, additive effects were observed with

10 organophosphate pesticides--diazinon, EPN, guthion, malathion, methyl parathion, OMPA, parathion, phosdrin, systox, and trithion. In a similar study in rats, Keplinger and Deichmann [48] obtained values for ratios of predicted over observed toxicity, based on LD50 after oral administration, of 1.30-1.82 for 4 organophosphate pesticides--diazinon, parathion, delnav, and malathion--administered with carbaryl. The authors concluded that these ratios suggested more than an additive effect. However, it is doubtful that this degree of increase in toxicity as evidenced by decreases in LD50's is sufficiently marked to be considered a synergistic or potentiating effect.

(2) Inhalation toxicity

Studies involving the exposure of animals to airborne carbaryl are relatively limited. Carpenter et al [27] exposed six guinea pigs for 4 hours to an airborne carbaryl wettable powder (50%) of 15 μm average particle size at a concentration of 390 (344-722) mg/cu m. The authors noted that at this concentration there was a visible dense cloud of dust. The animals gained weight normally during the subsequent 2-week observation period; however, nasal and local ocular irritation were evident. Autopsy disclosed healed hemorrhagic areas in the lungs. The authors provided no additional information.

Six guinea pigs were exposed for single 4-hour periods at a mean concentration of 230 mg/cu m of a "microfine" (average particle size was 5 μm, ranging from less than 1 to 10 μm) wettable powder containing 85% carbaryl by weight. [27] In the 14-day postexposure observation period, the animals initially showed slight weight losses, but they had returned to their pretreatment weights by the end of the period. Another group of five

guinea pigs survived a 4-hour exposure at a mean concentration of 332 mg/cu m to the same dust. No other information was provided.

An unspecified number of dogs were exposed [27] to microfine wettable powder of carbaryl at concentrations of approximately 75 mg/cu m. Within 5 hours, typical signs of cholinesterase inhibition were seen. Also, repeated exposures to the same formulation did not cause death or other evident injury in rats that inhaled 10 (5-20) mg/cu m for 7 hours/day, 5 days/week, for a total of 90 exposures. In an unpublished report (C Carpenter, written communication, January 6, 1976), the results of the gross and microscopic examinations of tissues taken from rats in the inhalation study were described. No signs of treatment-associated gross or microscopic lesions were present in the rats exposed to carbaryl (Sevin 85S) by repeated inhalation at the concentration used.

Yakim [39] reported the results of exposures of cats to airborne carbaryl. Three groups of four cats each were exposed for one period of 6 hours to carbaryl dust at concentrations of 82 (80.2-83.7), 37 (31.7-42.2), and 20 (18.7-21.2) mg/cu m. Yakim [39] did not specify the particle size, method of sampling, or sex or age of the cats. No deaths occurred, although the highest concentration did cause signs of toxicity which disappeared after exposure ceased. The cholinesterase activities fell immediately after exposure to 39-55% and 53-71% in serum and erythrocytes, respectively, but returned to normal in two of three cats in 72 hours. The third cat showed a partial recovery of cholinesterase activity after 72 hours. Cats exposed to a carbaryl dust at 37 mg/cu m showed 23% serum and 41% inhibition of erythrocyte cholinesterase activity which returned to normal in 48 hours. Carbaryl dust at 20 mg/cu m caused reductions of

11-24% in serum and 15-28% in erythrocyte cholinesterase activities, but recovery was complete in 24 hours. The authors concluded that the single exposure at 20 mg/cu m represented the threshold exposure concentration. Three additional groups of four cats each were exposed repeatedly to carbaryl dust. The first group, at an airborne concentration of 16 (15.3-16.6) mg/cu m, showed no signs of toxicity during the 4-month (6 hours/day) exposure. In the second group, at a concentration of 63 (61.1-64.2) mg/cu m for 6 hours/day for 1 month, periodic salivation was most profuse during the first 2 hours of each exposure. One cat died on the 20th day; the surviving cats showed reductions of 31-40% in serum and 41-58% in erythrocyte cholinesterase activities. The third group was exposed to carbaryl at an average concentration of 40 (37.4-43.3) mg/cu m for 6 hours/day for 2 months. Details were not given, but the author stated that some deterioration occurred in undefined conditioned reflexes of cats at this concentration, mostly after the first exposure; although erythrocyte cholinesterase activity dropped to 50% or less on some days, the conditioned reflexes remained normal. The author concluded that results from inhalation experiments of 1-4 months suggest that 16 mg/cu m constitutes the threshold concentration and 63 mg/cum is the toxic concentration of carbaryl for cats. Based on his studies on cats and humans which were discussed earlier, the author suggested that the maximum permissible concentration in the USSR be set at 1 mg/cu m. The author did not describe the method used to determine cholinesterase activity.

(3) Local (skin and eye) effects

Carpenter et al [27] also reported on studies investigating the possibility of injury to the surface of the eye following contact with

carbaryl. They applied 0.5 ml or more of various concentrations of an undescribed form of carbaryl to one eye of each of five rabbits. The eyes were examined for any immediate local effect; any 24-hour reactions were recorded after application of a 5% aqueous fluorescein stain to reveal the presence of injured tissue. Volumes of 0.5 ml or more as a 10% suspension of technical grade carbaryl caused only mild injury in one of five rabbit eyes at the 24-hour examination. A 25% aqueous suspension of "microfine" carbaryl (a powder containing 85% active agent by weight) with an average particle size of 5 μm caused no injury. Fifty milligrams of carbaryl dust caused only traces of corneal necrosis.

Yakim [39] found that application of a 10% suspension of carbaryl and of 50 mg of powder to the eyes of rabbits caused only transient miosis and hyperemia. Those signs could occur after local absorption of an anticholinesterase agent.

Carpenter et al [27] evaluated the primary skin irritation potential of topically applied carbaryl. A solution of technical grade carbaryl was applied at a concentration of 10% in acetone at a dose of 0.01 ml to the clipped abdominal skin of five rabbits. The authors noted that, whereas most of the carbaryl went into solution, a slight turbidity remained. The rabbits were observed for 24 hours; no signs of irritation were noted.

Gaines [49] investigated the acute dermal toxicity of carbaryl in rats. He applied technical grade carbaryl dissolved in xylene to cleanly clipped areas of skin over the shoulders and forward parts of the backs of 10 male and 10 female rats. The animals were at least 90 days old and ranged in weight from 175 to 200 g. The dermal LD50 values exceeded 4,000 mg/kg in both sexes. Gaines [49] stated that an LD50 by the dermal route

is much more indicative of the possibility of occupational toxicity than the acute oral LD50. Yakim [39] reported that, whereas no deaths occurred in six rabbits when a dose of 500 mg/kg of carbaryl was applied to the skin, serum and erythrocyte cholinesterase activities were inhibited during the first 24 hours after treatment. Yakim [39] also indicated that cholinesterase activity was normal after 72 hours and that the skin was not irritated. The cholinesterase activity was determined colorimetrically by Hestrin's method, [50] but no specific details were given on how the method was carried out. The original method by Hestrin [50] is not a reliable one for measuring cholinesterase activity in blood samples (see Chapter IV, Biologic Monitoring).

Carpenter et al [27] examined the skin sensitization potential of carbaryl. Sixteen male albino guinea pigs were treated with eight intracutaneous injections (3/week on alternate days) of 0.1 ml of a 0.1% carbaryl dispersion in 3.3% propylene glycol made up in 0.75% NaCl. After 3 weeks during which no injections were given, a similar dose was again administered intracutaneously, and all sites of injection were examined 24 and 48 hours after this challenge dose. The authors reported that 4 of the 16 showed evidence of weak sensitization.

(4) Other effects

Carpenter et al [27] fed carbaryl at 1,500 and 2,250 ppm in the diet to two groups of 10 rats each for 96 days. The higher level produced a decrease in the body weights of females, an increase in the liver-to-body-weight ratio in males, and an increase in the kidney weight of females compared with the controls. Food intake was not affected. The only microscopic finding was a minor degree of diffuse, cloudy swelling of

the kidney tubules in 4 of 10 animals fed 2,250 ppm of carbaryl. At the 1,500-ppm dose level, an increase in kidney weights of females was the only deviation from control values; organ damage was not evident upon microscopic examination. The authors speculated that the apparent difference in response between sexes occurred because the dose for females, in mg/kg of body weight, was about 30% greater than that for males at the same dietary intake.

Carpenter et al [27] reported a 2-year study in which rats received carbaryl mixed with feed. Five groups of 40 animals (20 males and 20 females), 60 days old at the beginning of the study, were administered 0, 50, 100, 200, and 400 ppm carbaryl in the diet. Before the start of the study, 10 of the male rats (age 54 days) were selected randomly from each of the 200- and 400-ppm treatment levels and from the control group for hematocrit determinations, which were made initially and at various intervals throughout the study. All surviving animals were killed between days 732 and 736 of the experiment. Gross and microscopic examinations were performed on gastrocnemius muscle, sciatic nerve, lung, kidney, liver, heart, spleen, pancreas, stomach, duodenum, descending colon, testis or ovary, fallopian tube, esophagus, trachea, thyroid, urinary bladder, and adrenal gland tissues. Lung infections, which accounted for 88% of all deaths during the study, appeared to be no more frequent among treated than among control rats. The other causes of death, none of which appeared to be dose related, were peritonitis, 6.5%; neoplasms, 3.3%; anuria, 1.1%; and nephritis, 1.1%. No single type of tumor or site of origin was associated with the inclusion of carbaryl in the diet, nor was the incidence of tumors in the carbaryl-fed groups different from that for control rats. In fact,

mortality in the control group exceeded that in any of the treated groups. No statistically significant difference was found between the rats fed carbaryl and the control group in mean age at death, mean expectation of life at birth, or mean expectation of life after 1 year of administration. The mean age in days at death for both sexes at the 200- and 400-ppm diet levels were 630 and 656 days, respectively, and 585 days for the control rats. Liver and kidney weights which were calculated as percentages of body weights of rats killed periodically during the experiment did not differ from control values. None of the mean hematocrit values for the 400-ppm diet-fed rats differed at any interval from those for the control group, and only a single midexperiment mean for the 200-ppm diet group was different from the controls. No significance was attached to this isolated deviation. Microscopic examination of tissues from randomly selected animals fed carbaryl in the diet for 180 and 270 days revealed no significant difference from the controls. Kidney changes of a mild, transitory nature observed in female rats fed 400-ppm carbaryl diet for 365 days were characterized as a cloudy swelling of the proximal convoluted tubules. After 2 years of receiving 400-ppm of carbaryl in the diet, some rats developed renal changes consisting of cloudy swelling of the proximal convoluted and loop tubules of the kidney, but the incidence was not significant. The authors considered the cloudy swelling of the hepatic cords, principally located around the central veins, to be more noteworthy. This was a finding of statistical significance in the group of animals on the 400-ppm diet for 2 years. Microscopic examination of rats at the lower doses, ie, 50, 100, and 200 ppm, revealed no differences between the treated and control animals. The authors concluded that after two years

none of the tissues examined from the rats on the 400-ppm diet showed permanent degenerative changes which could be attributed to toxicity of the insecticide.

Aspects of the comparative toxicology of carbaryl in rats and monkeys were presented in an abstract by Serrone et al. [51] The authors concluded that monkeys tolerated much larger single oral doses of carbaryl (up to 1,000 mg/kg) than did rats or dogs. Although monkey plasma cholinesterase was inhibited at a dose of 600 mg/kg in a 6-month study (number of doses and frequency not stated), little inhibition occurred at lower doses. The authors also noted that electron microscopic studies of the kidneys of rats and monkeys treated with carbaryl disclosed a marked vacuolation of the epithelium in the proximal renal tubules. In a review article which discusses the same or similar studies, Coulston and Serrone [44] presented an electron photomicrograph of renal tissue from a monkey treated with carbaryl at 600 mg/kg (length and frequency of treatment not stated) that also demonstrated this lesion. In the abstract, [51] the authors also reported having observed no disturbances in urinary function other than a discoloration of the urine (this was also reported in swine [52]), which the authors thought might be caused by the presence of a metabolite of carbaryl. It is difficult to draw any conclusions from these studies, [44,51] since the length and frequency of treatment was not specified and only a single electron photomicrograph was presented.

Carpenter et al [27] administered carbaryl in gelatin capsules to Basenji-Cocker dogs for 1 year. A control group received no carbaryl. Fourteen dogs were randomly distributed by sex and litter among the treated and control groups. Carbaryl was administered 5 days/week at doses of

approximately 0.45, 1.8, and 7.2 mg/kg. Hematocrit, hemoglobin, erthyrocyte fragility, and differential leukocyte evaluations were made prior to the initial dose and after 3, 6, 7.5, 9, and 12 months of administration. Except at 7.5 months, determination of BSP retention and serum alkaline phosphatase, urea nitrogen, and bilirubin concentrations were made at the same intervals. Plasma and erythrocyte cholinesterase determinations were also made five times during the 3-week pretreatment period, as controls. Twenty additional cholinesterase assays were performed electrometrically weekly for 9 weeks, twice during the next month, and about monthly thereafter. After a year of treatment, the dogs were anesthetized and exsanguinated. Brain, spinal cord, thorax, abdomen, gastrocnemius muscle, and sciatic nerve were examined. One of two bitches that received carbaryl at 0.45 mg/kg had shown transient hind leg weakness after the 189th day of administration. Carbaryl treatment was continued without pause and, within 21 days, the dog appeared to be normal. There was no statistically significant difference between treated and control animals with respect to mean body weight, mean blood values (hematocrit, hemoglobin, and differential leukocyte count), BSP retention, serum urea nitrogen, total serum bilirubin, and serum alkaline phosphatase values. Cholinesterase activity was not significantly different in the treated and in the control dogs; however, the method used to determine cholinesterase activity probably underestimated the degree of inhibition. The weights of livers and kidneys of the carbaryl-treated and control animals also did not differ. Microscopic examination revealed diffuse, cloudy swelling of the proximal convoluted and loop tubules of the kidney and focal sudanophilic "dust" in the glomeruli of dogs given carbaryl at 7.2 mg/kg. The authors

judged these to be transient conditions rather than the early stages of toxic degeneration, because the microscopic findings were also present in the control dogs, but to a lesser extent. Although considerable intracellular fat was observed in the proximal kidney tubules of the females, the authors did not regard this as indicative of toxicity, but rather as a variability within the normal range. Carpenter et al [27] concluded that tissues from the dogs killed after 1 year of carbaryl administered orally in doses of 7.2 mg/kg or less showed no permanent degenerative changes.

(b) Paralytic Effects

Grob, [53] in a review of neuromuscular pharmacology, indicated that the only residual effects after exposure to some organophosphate cholinesterase inhibitors were a few instances of paralysis. These disorders resembled the peripheral neuritis and demyelination (Ginger Jake paralysis) which occur at 14 days or more after exposure to triorthocresylphosphate (TOCP). Consequently, cholinesterase-inhibiting compounds are frequently tested for nerve-demyelination potential early in their development.

Carpenter et al [27] studied in chickens (Rhode Island Red hens) the potential of carbaryl to cause the type of paralysis referred to as Ginger Jake paralysis seen with TOCP. The chemicals (carbaryl and TOCP) were suspended in lard and single doses administered subcutaneously to chickens (13 carbaryl- and 10 TOCP- treated) at doses of 0.25, 0.5, 1.0, 2.0, and 3.0 g/kg (number of hens at each dose level unspecified). Five control hens were untreated, two hens were vehicle-treated (lard), and one additional hen was given undiluted TOCP at 1.0 mg/kg. Chickens that

received 1.0 g/kg or less of carbaryl did not develop signs of leg weakness. At 2.0 g/kg, leg weakness was observed on the first or second day following administration; in one case, the chicken was unable to walk for 3 days, but none developed late paralysis (after 14 days) typical of TOCP-demyelinating injury. All chickens given TOCP at 3.0 g/kg showed leg weakness at 14 days which persisted until they died. Microscopic examination of tissue sections of brain, sciatic nerve, and spinal cord revealed no evidence of demyelination at any carbaryl dose level; however, demyelination was present in some TOCP-treated chickens. The authors concluded that leg weakness was evidence of a transient cholinergic effect caused by the slow absorption of carbaryl from the subcutaneous depot. Evidence of slight degeneration (focal loss of striations and fatty infiltration of gastrocnemius muscle fibers) was present at the 3.0 g/kg dose of carbaryl and at all doses of TOCP. These authors concluded that the role of carbaryl in the production of leg weakness in chickens may be described as cholinergic rather than demyelinating.

Gaines' study [43] in chickens supports the studies of Carpenter et al. [27] Gaines pretreated an unspecified number of chickens with 15 mg/kg of atropine orally to protect against the acute effects of the subcutaneous administration of 800 or 1,600 mg/kg of carbaryl. The animals were then observed for 21 days. The higher dose of carbaryl caused leg weakness within 24 hours; all chickens recovered by day 24. As stated above, in TOCP poisoning paralysis develops after 14 days and continues until death.

Smalley et al [52] fed a ration containing carbaryl to 13-week-old Yorkshire pigs. One male and one female pig received 150 mg of carbaryl/kg/day for 72 and 83 days, respectively; one female and two male

pigs received 150 mg/kg/day for 28 days followed by 300 mg/kg/day for either 18 (both males) or 57 (one female) additional days. Two pigs from the same litter served as controls. During the first 4 weeks of treatment, signs of carbaryl effect were limited to brown discoloration of the urine upon exposure to light and air. The first group (one sow and one boar) that received 150 mg/kg/day showed signs of toxicity on days 45 and 62 respectively. The second group (2 boars and 1 sow), whose dose was increased from 150 to 300 mg/kg/day on day 29, exhibited signs of toxicity on days 37, 39, and 41. Animals in both groups were reluctant to stand, remaining recumbent for long periods; later, they were ataxic, incoordinated, and had tremors. In the first group, the boar became prostrate and died on day 72; the sow became prostrate on day 80 and died on day 83. In the second group, both males were prostrate by days 43-44 and died on day 46, while the female became prostrate on day 71 and died on day 85. Microscopic examination of skeletal muscle revealed three distinctive types of myodegeneration, one type related to trauma or ischemia, another characterized as hyaline and vacuolar degeneration, and a third type associated with dystrophic calcification of mitochondria and the sarcotubular system. In the myelinated tracts of the cerebellum, brain stem, and upper spinal cord, the moderate-to-severe edema was considered by the authors to be caused by vascular changes characterized by endothelial hypertrophy, hyalinization of vessel walls, and widespread hemorrhages; however, no demyelination of nerve tissue was observed. In another publication, Smalley [54] indicated that administration of hydrochlorothiazide, a diuretic, reversed the signs of toxicity of carbaryl in chronically treated pigs. He suggested that the mechanism of this

reversal was related to increased excretion of carbaryl in the urine. In the same study, two 5-mg doses of atropine given intramuscularly, 2 hours apart, proved effective in controlling signs of acute carbaryl intoxication in pigs administered single oral doses of carbaryl at 2 g/kg. One sow which received carbaryl in the diet at a dose of 150 mg/kg/day developed paresis on day 93. Atropine therapy, 5 mg (im) repeated at 8-hour intervals for a total of 40 mg over a 3-day period was not effective in controlling signs resulting from repeated administration of carbaryl. The author stated that the only unusual gross or microscopic lesions were in the striated muscle, and that these lesions resembled myopathies of toxic or nutritional origin. The author suggested that brown urine might be evidence of carbaryl poisoning, but it may have actually indicated only carbaryl absorption.

The sensitivity of the pig to carbaryl was compared with that of the dog in a study by Miller et al. [55] A single 20-mg/kg dose of carbaryl was injected iv into eight male miniature pigs. Four control pigs received the vehicle only, which consisted of peanut oil, saline, and lecithin. Both control and experimental pigs were killed 20 minutes after injection. Five male pigs had been fed carbaryl at 125 mg/kg in the diet for 6-8 weeks and were then killed, and five served as controls. Five male dogs received carbaryl at 30 mg/kg by single iv injection. Five additional dogs received the same vehicle as the pigs and served as controls. Both control and experimental dogs were killed 20 minutes after injection. Six male dogs received carbaryl at 125 mg/kg in the diet for 2 months, and five additional dogs were used as controls. All eleven were killed at the end of the study. The signs of carbaryl toxicity following iv injection were

more marked in the pigs than in the dogs. Tremor, ataxia, incoordination, and paraplegia were observed in the pigs, but only lacrimation, salivation, and occasional tremors occurred in the dogs. Dietary administration failed to produce any overt signs of toxicity in the dog, whereas a spastic paresis of the posterior extremities developed in the pigs after 6-8 weeks on the carbaryl diet. There was a significant depression of brain cholinesterase of a similar degree in each species, but the method may not have adequately detected cholinesterase inhibition.

(c) Behavioral Effects

Santolucito and Morrison [56] examined the effect of carbaryl on the EEG of the rhesus monkey. Daily oral carbaryl doses of 0.01 and 1.0 mg/kg were given to four and three rhesus monkeys, respectively, for 18 months; seven monkeys served as controls. During that period, the experimental animals showed no obvious changes in behavior. After 18 months, a single 15-minute EEG recording was made from each monkey while they were immobilized and anesthesized. There were no significant quantitative EEG changes, but whether this was due to anesthetic suppression is not known.

Sideroff and Santolucito [57] conducted a series of investigations on the effects of carbaryl on rat behavior. Two techniques, liquid reinforcement and electroshock avoidance, were combined and used in this study. Each of four groups of male rats weighing 200-250 g was composed of 5-9 controls and an equal number of treated animals which were injected subcutaneously with carbaryl at 10 mg/kg, once weekly, for 2-5 weeks. The results of the experiments showed a statistically significant difference between the control and the treated animals. This led the authors to conclude that the carbaryl-treated rats were less motivated (fewer lever

pressings for water) and less inhibited by aversive (electroshock) situations than were the control rats.

Singh [58] found that a decrease in physical activity of rats, as measured in an activity-wheel cage, occurred following single ip injections of carbaryl at 0.56 and 2.24 mg/kg. Atropine at 2 mg/kg ip did not alter the carbaryl effect in female rats. In male rats, the effects of the 2.24 mg/kg dose were reversed.

(d) Reproduction Studies, Including Teratogenesis and Mutagenesis

Epstein et al [59] included carbaryl in a series of agents screened for dominant lethal-mutations in mice. Carbaryl was administered orally in daily doses of 50 and 1,000 mg/kg to 10 male mice each for 5 days. One mouse in the 1,000 mg/kg group died. Each of the 19 surviving males was then caged for mating with three untreated virgins each week for 8 consecutive weeks. The females were killed on about the 13th day of gestation. At autopsy, each female was scored for pregnancy and for number of total implants, including live implants and early fetal deaths. The authors assessed the reduction in total implants by comparing the number of total implants in females mated with treated and control males. While these specific data for carbaryl were not discussed further in the paper, the authors included carbaryl in a class of agents which did not meet "any screening criteria for mutagenic effects."

In addition to the dominant-lethal test, the results of several other studies of the mutagenic potential of carbaryl have been reported. In a plant study undertaken by Amer, [60] 0.5 and 0.25% saturated solutions of carbaryl (pure and formulated), prepared at 22 and 60 C, were applied to Allium cepa germinating roots for 4 and 24 hours. The pure and formulated

solutions prepared at the higher temperature (60 C) caused abnormal forms of mitotic figures and a complete arrest of the process. Solutions prepared at the lower temperature caused fewer abnormal forms of mitotic figures and partial arrest of the mitotic process. The formulation caused more severe arrest than did the pure solutions. The relevance of these data to humans is not clear at this time.

In another plant study, Wuu and Grant [61] using barley (Hordeum vulgare L) seeds and seedlings found that carbaryl at 1,000 ppm for 12 hours on the sprouted seeds induced meiotic changes, namely, chromosomal aberrations of 0.55% in the C-1 generation and 2.9% in the C-2 generation. The positive controls, treated with ethylmethanesulfonate at 1,000 ppm and X-rays at 5,500 R, and the untreated control sprouted seeds showed chromosomal abnormalities of 1.57%, 3.91%, and 0.22%, respectively, in the C-1 generation and 0.38%, 1.26%, and 0.49%, respectively, in the C-2 generation. The seedlings sprayed once with carbaryl at a concentration of 500 ppm which showed 1.21% chromosomal abnormalities contrasted with the untreated control rate of 0.66%.

Brzeskij and Vaskov [62] examined the effects of carbaryl on mutations and fertility in Drosophila melanogaster using criteria of (1) deletions in X-chromosomes, (2) recessive sex-linked lethal and sublethal mutations caught in F2 by Moeller-5 method, and (3) male fertility by examining sex cells in different stages. They found no effects on fertility of males at any stage of spermatogenesis but found a low rate of recessive sex-linked lethal and sublethal mutations. Therefore, they concluded that carbaryl was a weak mutagen. Elespuru et al [63] found that carbaryl was not mutagenic in Hemophilus influenzae. Siebert and

Eisenbrand [64] examined carbaryl for genetic activity in a diploid strain of Saccharomyces cerevisiae, heteroallelic at the gene loci ade-2 and trp-5. Even at a concentration of 4.97 millimolar, carbaryl did not change the frequency of mitotic gene conversion compared with control solvents and was judged genetically inactive. Uchiyama et al [65] assayed carbaryl for mutagenic activity by using (1) the back-mutation method with an auxotrophic mutant of E coli B/r WP-2 try and (2) recombination assay with B subtilis Marburg 17a, of recombination-capable strain, and Marburg 45T of recombination-lacking strain. Carbaryl did not result in mutagenic changes, even at the highest concentration of 10 mg/plate, with either method.

The preceding mutagenic studies on a mammal (mice), [59] on bacteria (H influenzae, [63] and E coli and B subtilis [65]), and on yeast (S cervisiae) [64] indicate that carbaryl is not a mutagen under these experimental conditions, although experiments on an insect (Drosophila) indicate weak mutagenicity. [62] However, nitrosocarbaryl (N-nitroso-N-methyl-1 naphthylcarbamate) was a strong mutagen in H influenzae, [63] S cerevisiae, [64] and E coli and B subtilis. [65] Uchiyama et al [65] also pointed out that the N-methyl carbamates, including carbaryl, might be convertible to nitroso compounds. They speculated that since carbamates are widely used and since nitrite is a common constituent of human saliva, the stomach would then provide the acid medium necessary for nitrosation. Nevertheless, the significance of the formation of nitrosocarbaryl in relation to any effect of carbaryl on humans remains to be determined.

Smalley et al [66] investigated the teratogenic potential of carbaryl incorporated into the diet of pregnant beagles throughout gestation

(averaging 62 days). Fifty-five bitches were treated with carbaryl as follows: 10 at 3.125 mg/kg, 10 at 6.25 mg/kg, 18 at 12.5 mg/kg, 9 at 25 mg/kg, and 8 at 50 mg/kg. Sixteen animals used as controls were maintained under similar conditions but received no carbaryl. After parturition, the bitches and pups were carefully examined. Radiographs were taken of the pups if skeletal abnormalities were observed or suspected. Defective pups were killed and necropsied after birth, the rest at weaning; abnormalities were characterized and recorded. At all levels of treatment, the percentage of pups born alive was lower than in the controls. At the highest carbaryl dose (50 mg/kg/day), eight animals were bred, three conceived, but no pups were born alive. Dystocia was present in about one out of three of the treated bitches; none of the control bitches had dystocia. The authors did not state whether the dystocia was maternal or fetal but, from the information given on the appearance of the reproductive organs, it is inferred that the dystocia was maternal. The average numbers of pups/litter at the two highest dose levels were 3.5 and 3.8; the control average was 5.4. Of the total number of pregnant beagles at all carbaryl doses, 21% produced pups showing evidence of embryotoxicity. At the lowest dose (3.125 mg/kg) and in the control group, there were no abnormal pups. Excluding fetal deaths and resorptions, 21 pups (11.6%) were abnormal; all were from dams that received the four higher doses. Teratogenic effects included abdominal-thoracic fissures with varying degrees of intestinal organ agenesis, extra phalanges, brachygnathia (shortened lower jaw), and failure of skeletal formation. This study [66] was listed in the Registry of Toxic Effects of Chemical Substances [14] as showing teratogenic effects and will be discussed and evaluated further (see Absorption and Metabolism

under Animal Toxicity, Correlation of Exposure and Effect, and Chapter V, Basis for the Recommended Standard).

In studies [67] designed to evaluate the effects of carbaryl on reproduction in primates, carbaryl was administered in 1% aqueous gum traqacanth by stomach tube to mature female rhesus monkeys throughout the gestation period, as follows: 2.0 mg/kg to 4 monkeys and 20.0 mg/kg to 10 animals. Seven control monkeys received the vehicle. Monkeys at all treatment levels, including controls, were mated and confirmatory pregnancy tests performed. Two of four monkeys given carbaryl at 2.0 mg/kg were pregnant but both aborted. Six of 10 monkeys on the 20 mg/kg dose were pregnant; 3 aborted and 3 delivered normal babies. Five of seven control monkeys were pregnant; one aborted, and four delivered normal babies. Although not directly dose related, abortion rates for the treated groups--100% (two of two) at 2 mg/kg, 50% (three of six) at 20 mg/kg--exceeded the 20% rate (one of five) for the control group. It is difficult to make conclusions on the basis of this report since the number of monkeys used was too small. Abortions were reported but no terata were found; this agrees with Wilson [68] who has indicated that with several suspected or proved teratogens, the range of doses causing terata in the primate is small; moreover, unlike rodents, primates are more likely to respond to potential teratogens by aborting.

Dougherty and Coulston [69] extended the previous study [67] using 79 female rhesus monkeys; 78 finished the study. The animals were divided into 4 groups of 16 each and 1 group of 15 (vehicle control). Each monkey of three groups was given carbaryl in a gelatin capsule at 0.2, 2.0, or 20 mg/kg/day from day 20 through day 38 of gestation; the fourth group was

given empty capsules; the fifth group was not treated. All monkeys were mated and delivered naturally with the following results: the 0.2 mg/kg group had 14 live births and 2 abortions; the 2.0 mg/kg group had 15 live births and 1 abortion; the 20.0 mg/kg group had 12 live births, 3 abortions, and 1 was not pregnant; the vehicle control group had 13 live births, and 2 abortions; the untreated control group had 13 live births, 2 abortions, and 1 stillborn. No terata were found. The authors concluded that carbaryl was not teratogenic, that it was not associated either with a high incidence of abortion or stillbirth or with abnormal gestation length or mean body weight, and that it did not have any adverse effect on adult females or surviving infants. Microscopic examination of the tissues of the young monkeys which died was not performed.

Weil et al [70] reported a study of the teratogenic potential of dietary carbaryl in rats. After mating, pregnant rats were assigned at random to one of three treatment schedules: (1) carbaryl in the diet throughout pregnancy or until weaning of the pups; (2) carbaryl in the diet from days 1 to 7 of pregnancy; and (3) carbaryl in the diet from days 5 to 15 of pregnancy. Females for each of the three treatment schedules were randomly assigned to three groups of six rats each and administered 20, 100, or 500 mg/kg of carbaryl. Six rats were assigned to the control group. Neither fertility nor gestation was affected by carbaryl in the diet. However, at the dose of 500 mg/kg until weaning, 2/3 of the pups died within 4 days after birth. The viability of pups in the other treated groups was not significantly affected. The mean body weights of the pups from control and treated dams were similar, although the adult female rats and their pups that received 500 mg/kg of carbaryl during pregnancy and

nursing weighed less than did the control dams and pups at time of birth. No gross or teratogenic anomalies associated with carbaryl treatment were found in the pups weaned at 21 days of age. Six adult rats of each of the nine treatment groups and one control group were killed on days 19-21 of their pregnancies. The uteri were examined for resorption sites and for dead and living pups. Gross and microscopic examination revealed no significant abnormalities attributable to carbaryl in the 709 fetuses examined. The poor survival of the rat pups allowed to nurse may have been related to the excretion of carbaryl or its metabolites in the milk and to the greater sensitivity of young rats to carbaryl, or it may have been related to the inability of the dams treated with carbaryl at 500 mg/kg to successfully nurse and rear their young, as indicated by their limited body weight gains.

The teratogenic potential of carbaryl has also been investigated in various other species of animals. Robens [71] administered carbaryl to guinea pigs daily in gelatin capsules on days 11-20 of gestation at a dose of 300 mg/kg. This resulted in 38% mortality in 26 pregnant dams, as compared with no deaths in control guinea pigs given empty gelatin capsules. Fetal mortality was 17.5% in the litters of surviving treated dams and 9.5% in control litters. There were 11 terata among the fetuses of these treated dams. The changes included skeletal defects, most of which involved the cervical vertebrae. Carbaryl was also administered as a single-dose treatment of 300 mg/kg to another group of 40 guinea pigs, some (number not specified) of which received their single doses between days 11 and 20 of gestation. Maternal mortality for this group was 12.5%; and fetal mortality was 6.5%, which was below that for the control group

(9.5%). All nine malformed fetuses were produced in the litters of dams treated on days 12, 13, 14, 15, and 16. Eight of the nine had vertebral malformations, and two revealed organ agenesis. One of the two malformations in control fetuses was dental, the other vertebral. The mortality of the adult females in all groups which produced terata, was greater than in the controls. Robens [71] stated that the doses required to produce terata in guinea pigs were at least 1,000 times the level of carbaryl allowed in human food. This study [71] was one of those listed in the Registry of Toxic Effects of Chemical Substances [14] as showing teratogenic effects, and will be further discussed later.

Oral administration [71] of carbaryl in gelatin capsules at doses of 50, 100, and 200 mg/kg to four, four, and nine pregnant rabbits, respectively, on days 5-15 of gestation produced neither terata nor dose-related fetal mortality, as compared to the results from 21 pregnant control does. The author indicated that signs of cholinesterase inhibition did not occur following any dose of carbaryl used in this study.

Carbaryl was administered by stomach tube to pregnant hamsters [71] on days 6, 7, and 8 of gestation at a dose of 125 mg/kg, or on day 7 or 8 at 250 mg/kg. Two of six hamsters treated with one dose of 250 mg/kg of carbaryl died, none of the eight hamsters given 125 mg/kg died, and none of the controls. Signs of cholinesterase inhibition (salivation, diarrhea, and incoordination) were observed in all treated hamsters. Fetal mortality was 30.3% at the high dose (250 mg/kg), 10.0% at the low dose (125 mg/kg), and 5.5% in the controls. No anomalies were found in any of four fetuses examined from each litter. The results show that, among the species studied, [71] terata were produced only in guinea pigs and by doses which

also caused mortality and morbidity in some of the dams. Also, since only one level was used in the single- and multiple-dose guinea pig studies, no conclusions of dose-related significance can be made.

In another study by Weil et al, [72] 300 pregnant guinea pigs in groups of 5 or 10 were administered doses of 100, 200, or 300 mg/kg of carbaryl by dietary inclusion, or 50, 100, or 200 mg/kg by gastric intubation, for 1-, 2-, 3-, 5-, or 15-day intervals during days 10-24 of gestation and were killed prior to parturition on day 34 or 35. Two control groups of 10 and 30 guinea pigs were treated in the same manner as the dietary- and gavage-treated guinea pigs, respectively. Body-weight gains for all guinea pig dams treated on days 12, 13, 14, 15, and 16 were less than those of the control animals, and this effect was more marked in intubated than in diet-treated guinea pigs at the same dose. Fewer carbaryl-gavaged than control-gavaged pregnant guinea pigs died. Dietary carbaryl produced no dose-related incidence or significant numbers of terata, as compared with controls. Oral intubation of carbaryl at 50 mg/kg to pregnant guinea pigs on days 10-24 of gestation produced 15.7% fetal skeletal anomalies compared with 9.1% for control pups, while doses of 100 and 200 mg/kg resulted in 5.4 and 8.3%, respectively. The authors [72] contrasted their results with those of Robens [71] and Shtenberg and Ozhovan [73] (discussed later in this section) who used gastric intubation as the route of administration, thereby causing greater toxicity than when compounds are incorporated in food. Weil et al [72] concluded that a carbaryl dose of 300 mg/kg in the diet or 200 mg/kg by intubation did not result in teratogenic effects in guinea pigs. They questioned whether the material used in the USSR study [73] was sufficiently pure. Argauer and

Warthen [74] have subsequently shown contamination of samples of carbaryl manufactured outside the United States (see later discussion in review of Carcinogenesis).

Benson et al [75] investigated the teratogenic potential of carbaryl in mice. Carbaryl was administered in the diet at levels of 10 or 30 mg/kg to groups of 20 pregnant mice from day 6 of gestation until birth of the pups. Twenty untreated females served as controls. The treated and control adults did not differ in mortality, behavior, physical condition, resorptions, or fetal deaths. The fetuses from treated dams did not differ from control fetuses in mortality and weight, but nine so-called minor fetal abnormalities in two litters occurred at the high dose (30 mg/kg), compared with two for the controls. The authors concluded that, because of the small incidence of abnormalities (6% at 30 mg/kg vs 2.1% in controls) and the lack of a consistent pattern in those found, the abnormalities were not related to administration of carbaryl. Lack of sufficient detail in their report makes an analysis for an independent evaluation impractical. However, lack of graded response in various abnormalities reported, at doses of 0, 10, or 30 mg/kg, supports their conclusion.

Vashakidze [76] carried out a 3-month study on rats at oral doses of 50, 100, and 300 mg/kg to measure the reproductive effects of what was described as Sevin powder. At doses of 100 and 300 mg/kg administered to female rats, there was a disruption in the sexual cycle reported as a decreased frequency or absence of estrus, proestrus and diestrus as well as elongation of the latter two phases. At the same doses, treated females impregnated by untreated males were reported to have reduction in litter size and prolonged pregnancy with what was described as deformation of the

womb. Autopsy showed embryos either dead or at various stages of development. The author reported that 50 mg/kg of the compound administered during organ differentiation, which was reported to be on the 9th or 10th day of development, caused developmental disorders. These disorders were explained in the study as either the death or cessation of development of the embryo. At unspecified doses, males were reported to show decreased sperm motility and deformed spermatozoa. At 300 mg/kg, treated males were unable to impregnate untreated females. It is not possible from the study described by Vashakidze [76] to determine either the purity or chemical composition of the material administered to rats that was described by the author as Sevin powder or the method of oral administration. Since the study was purely descriptive with no data presented, the results cannot be adequately evaluated. This study, unevaluated, was one of those listed in the Registry of Toxic Effects of Chemical Substances [14] as showing teratogenic effects.

Shtenberg and Rybakova [77] administered carbaryl described as 100% active material orally at doses of 7, 14, and 70 mg/kg to groups of 24 rats of each sex for periods of up to 12 months. A group of equal size received no carbaryl. The method of administration, whether by dietary inclusion or by stomach tube, is not clear from the text description. When compared with control rats, growth was inhibited in those receiving 14 and 70 mg/kg of carbaryl, but not in those receiving 7 mg/kg. Cholinesterase activity in blood as measured by acetylcholine-hydrolysis time was decreased in the 14 mg/kg and 70 mg/kg groups from the third month (first determination) until the end of the study. After 12 months of treatment, there was a dose-related decrease in spermatozoal motility at all doses. Microscopic

examination of the testes showed edema of interstitial tissue, destruction of germinal epithelium, and reduction in spermatocytes and spermatids. In females, the estrus cycle was prolonged by an increase in length of the diestrus phase at the 14 mg/kg and 70 mg/kg doses. All groups of carbaryl-treated rats showed an increase in gonadotropic hormone production in the hypophysis as determined by tests on immature mice. Decreased thyroid activity was indicated by a reduction in the absorption and excretion of 131-I. When examined microscopically, the cortex of the adrenal glands also showed evidence of increased activity in the zona glomerulosa and zona fasciculata. The authors suggested that the primary site of action was probably the anterior pituitary which caused secondary changes in the reproductive glands; however, a direct effect of carbaryl on the reproductive glands was not ruled out.

Collins et al [78] studied the effects of carbaryl on the reproductive cycle in a three-generation reproduction study in rats. Carbaryl (technical grade, 99% purity) was administered by incorporation into the diet at levels of 0, 2,000, 5,000, and 10,000 ppm and was continued throughout the study. Groups of 20 pairs of weanling rats at each dose plus the same number of untreated controls were mated at 100 days of age. Two litters (F1a and F1b) were produced from each pair. Animals from the first litter (F1a) were reared to weaning and then killed. Those from the second litter (F1b) were raised to weaning and 20 littermate pairs were selected to produce the next generation. The same procedure was followed until two litters had been produced for each of three generations (F1a, F1b, F2a, F2b, F3a, and F3b). All the rats of the third generation (first and second litters) were killed at weaning. The authors stated that

microscopic studies of the rat tissues were not performed. The ability of the female rats to produce young (fertility) was decreased only at the 10,000-ppm level. No litters resulted from the second mating of the second generation at this dose; therefore, no third generation was produced. The viability of the pups from dams treated at 5,000 and 10,000 ppm was significantly decreased. The average litter size and survival of offspring until 4 and 21 days of age were significantly decreased in a dose-related manner at both the 5,000-ppm and 10,000-ppm dietary levels. Mean weanling body weights at all carbaryl dose levels were significantly lower than those of control young and showed a dose-related suppression. The decreased fertility in rats on the highest dose was suggested by the authors as possibly due to an effect of carbaryl on sperm motility and the enzymatic activity of testes and ova, and mediated indirectly through effects on the hypothalamohypophyseal complex. The authors also suggested that the decreased survival rate of the 1- to 4-day-old pups, especially at the two higher doses, appeared to result from an increased susceptibility of the pups to metabolic damage from carbaryl treatment. Collins et al [78] concluded that the no-effect level for carbaryl was below 2,000 ppm.

A three-generation study of similar design was conducted in rats by Weil et al. [72] This study design differed from that of Collins et al [78] in that offspring of the first mating of the third generation (F3a), when killed at 21 or 90 days of age, were examined for tissue changes. The female rats used to produce the second litter of the third generation (F3b) were killed at day 18 or 19 of the second gestation period; examination of the uterine contents for viable or dead fetuses, resorption sites, fetal weight, and skeletal or soft tissue anomalies of the fetuses was performed

as in teratogenic studies. Some males of the second generation (F2a) were removed from treatment at 224 days of age and mated for 10 consecutive weeks to virgin females that had never received carbaryl, to test for dominant-lethal mutagenicity. The possible differences between gastric intubation versus dietary inclusion were also compared in this study. The dose levels for those rats treated by intubation were 0, 3, 7, 25, and 100 mg/kg, while the doses given in the diet were 0, 7, 25, 100 (also 100 in corn oil), and 200 mg/kg. Carbaryl at a level of 100 mg/kg by oral intubation produced signs of cholinesterase inhibition and increased mortality of parents at all breeding periods; it decreased the number of pups born alive (F1a, F2a, F3a) and the percentage of females that produced litters (F1b only); it decreased the number of fetuses and of live fetuses; it increased the frequency of fetal resorption; it lengthened the gestation period of female rats yielding first generation, second litter (F1b); and it decreased the body weights of the original parents before the first mating. The lower doses (3, 7, and 25 mg/kg) of carbaryl by oral intubation were without effect. The effects of 200 mg/kg of carbaryl administered by inclusion in the diet were limited to an initial decrease in body weight gain in the original rats and lengthened gestation periods of the first and second generations as compared with the controls. No signs of cholinesterase inhibition were observed at this dose level. The authors reported that the teratogenic and dominant-lethal portions of the study did not indicate any carbaryl-related effects. The results of this study, and of the previously discussed guinea pig teratogenic study included in this paper, [72] suggested that, at equal doses, carbaryl administered by stomach tube can produce a more pronounced toxic effect

than by dietary inclusion. This is probably due to the higher blood levels from the rapid absorption which can occur from gastric intubation.

Shtenberg and Ozhovan [73] gave carbaryl dissolved in sunflower oil perorally to rats (second (F2) through fifth (F5) generation) at doses of 2 and 5 mg/kg. According to their written communication to Weil and coworkers, [72] this was administered by gastric intubation. A control group was maintained under similar conditions. The test material was administered to both sexes of each generation for 6 months, but the rats were paired for breeding after 4 months of treatment. Sperm motility and resistance (not defined), spermatogenesis, and duration of sperm survival (in a nutrient medium) were significantly reduced in male rats of both treated groups from the second to the fourth generation when compared with the controls. Microscopic examination of testicular tissue revealed what was described as dystrophic-atrophic changes in the nature of the spermiogenic epithelium. In both the third and fourth generations, the duration of estrus was decreased and the interestral period lengthened in rats after 3 months of 5 mg/kg carbaryl treatment; at 6 months, rats receiving 2 mg/kg carbaryl were similarly affected. Microscopic examination of ovarian tissue revealed what was called an atrophically sclerotic process in the follicles of carbaryl-treated females. The authors stated that the effect was seen to increase from generation to generation. The dose of carbaryl at which these tissue changes were seen was not specified. Fertility of the females (litter size) decreased progressively from the second through the fourth generations, the effect being dose related. Survival of the pups during the first month of life decreased as the dose of carbaryl was increased. The adverse effect on

survival was seen to increase progressively from the third through the fifth generations. From the results of these studies, the authors concluded that carbaryl has a direct and negative influence on the reproductive glands.

Collins et al [78] conducted a three-generation study in Mongolian gerbils (Meriones unguiculatus). Carbaryl was administered in the diet at levels of 0, 2,000, 4,000, 6,000, and 10,000 ppm. No litters were produced from the second mating of the third generation (F3b) at the 10,000-ppm level. Adverse effects on fertility, litter size, pup viability, and survival to day 21 appeared sporadically in the second and third generations at carbaryl treatment levels of 2,000, 4,000, and 6,000 ppm, and in all generations at 10,000 ppm. Survival from day 4 to weaning was significantly decreased in all generations at doses of 4,000 ppm and above, while effects were seen in the second and third generations at 2,000 ppm. No grossly visible abnormalities were observed in any of the offspring. Microscopic examination (tissues not specified) revealed no changes. Mean weanling weights were variably lower in gerbils treated with 4,000 ppm or more of carbaryl.

(e) Carcinogenesis

Carpenter et al [27] investigated the lung cancer potential of carbaryl which was injected subcutaneously once a week into 60 male mice (tumor-susceptible A/Jax and C3H strains) during their third to eighth months of age, after which a gross examination only was made for lung tumors. Technical carbaryl was suspended in 0.25% agar at a concentration of 5.0%, and 0.2 ml was injected into each mouse, delivering a carbaryl dose of 10 mg/week to each mouse (or a dose of about 400 mg/kg/week in a 25

g mouse). One control group received injections of 0.25% agar only, and another was untreated. The authors stated that males of the A/Jax strain have a high rate of spontaneous lung tumor. Under the conditions of the experiment, subcutaneous administration of carbaryl did not significantly increase the incidence of tumors, lung infection, or death in tumor-susceptible mice over that in the controls.

Innes et al, [79] in a study of 120 compounds performed in conjunction with the National Cancer Institute, administered carbaryl to neonatal mice daily for about 18 months. The mice received the test material (4.64 mg/kg) by stomach tube on days 7-28 of age and thereafter in the diet at a level stated to be equivalent to the amount ingested. A total of 72 mice, 36 of each sex, composed the treatment group. Eleven of the compounds studied caused a significant elevation of tumors; 89 more compounds, including carbaryl, gave no significant evidence of tumorigenicity; the remaining 20 warranted, it was concluded, further evaluation. Further data on carbaryl-treated mice were not supplied. The authors pointed out that there was no way to predict whether humans are more or less susceptible than mice to the induction of tumors by the compounds tested in this study. In addition, they indicated that the dose received by the mice was far in excess of that likely to be consumed by humans.

Andrianova and Alekseyev [80] administered carbaryl orally to 60 male mongrel rats at 30 mg/kg twice weekly for periods of up to 22 months (whether by incorporation into their diet or by intubation is not clear). Another 48 mongrel rats were treated by subcutaneous implantation of a paraffin capsule containing 20 mg of Sevin (carbaryl) 97.65% pure obtained

from the Shchelkov Chemical Plant. The control group consisted of 48 male rats, but it was not stated whether they were sham-treated orally or had empty capsules implanted subcutaneously. Of the 12 surviving rats in the orally treated carbaryl group, 4 had tumors; there were 2 subcutaneous fibrosarcomas, 1 polymorphous cell sarcoma (the tumor grew into the stomach wall but did not affect the organs of the abdominal cavity), and 1 osteosarcoma. Of the 10 surviving rats in the subcutaneously treated group, 2 had subdermal tumors, both of them said to be fibrosarcomas, but not at the implantation site. In the control group, 46 of 48 rats survived, and only 1 had a tumor at 11 months, a fibrosarcoma at an unspecified location. Although the authors concluded that carbaryl could produce tumors in rats, the results of this study are not conclusive because of the high mortality in the experimental group and the absence of information on how the controls were treated. This study [80] was listed, unevaluated, in the Registry of Toxic Effects of Chemical Substances [14] as showing carcinogenic effects. It will be further discussed and evaluated (see Correlation of Exposure and Effect and Chapter V, Basis for the Recommended Standard). As previously mentioned in the discussion of reproduction studies, Weil et al [72] have questioned the chemical purity of the carbaryl produced outside the United States. The following paper may partially answer the question. Argauer and Warthen [74] analyzed various samples of carbaryl (1-naphthyl methylcarbamate) for the presence of the contaminant 2-naphthyl methylcarbamate by using liquid and thin-layer chromatography with confirmation by spectrofluorometry. Contamination of carbaryl with the 2-naphthyl methylcarbamate can occur if the precursor 1-naphthol, used to synthesize the carbaryl, is not pure.

The presence of the 2-naphthyl isomer is undesirable since the compound has been reported to have caused cancerous tumors in rats and in mice when given orally or intravenously. [74] Of the four samples of carbaryl produced in the United States which were analyzed in this study, none contained a detectable amount of the 2-naphthyl contaminant. Each of the four samples produced in foreign countries contained measurable amounts (2.3 and 14 mg in the 250-mg technical samples; 1.3 and 12.4 mg in the 500-mg samples of 50% wettable powder) of the 2-naphthyl isomer measured as 2-naphthol in a previously hydrolyzed sample. In the previously cited paper by Carpenter et al, [27] there was no dose-related incidence of tumors or increase in tumors over the controls in rats given US-manufactured carbaryl in the diet at levels of 50, 100, 200, and 400 ppm for 2 years.

Shimkin et al [81] synthesized N-methyl naphthyl carbamate and compared it to 21 other carbamates, one of which was ethyl carbamate, a known carcinogen, by a sensitive, pulmonary-tumor-induction bioassay method in Strain A/He mice from the National Cancer Institute. The compound was administered ip to 16 mice at 0.5 mg 3 times weekly for 4 weeks; the total dose to each mouse was 6.0 mg (1,190 μmoles/kg). Three control groups of mice (32 in each group) were injected with water or the vehicle tricaprylin or were untreated. The experiments were terminated 20 weeks after the last injection by killing the animals. The lungs were examined microscopically for tumors. While several of the carbamates were actively tumorigenic (ethyl carbamate, most active; methyl carbamate, inactive), the synthesized N-methyl naphthyl carbamate was classed as marginal. Two important points need to be emphasized concerning the compound described in the study of Shimkin et al [81] as N-methyl naphthyl carbamate. First, there is a lack

of information on the purity of this specially synthesized compound, and, secondly and more importantly, it is not clear from the structural formula presented whether the compound was 2-naphthyl N-methylcarbamate or 1-naphthyl N-methylcarbamate (carbaryl). Therefore, it is difficult to draw any conclusion as to the potential for carcinogenicity of carbaryl from this study. In a study using Ehrlich ascites tumor cells, injected intraperitoneally, Walker et al [82] determined that carbaryl, also injected ip, produced a moderate but significant inhibition of tumor growth in vivo in mice. The authors also found that carbaryl "reduced appreciably" the rates of incorporation of the isotopically labeled precursors, uridine-5-3H, thymidine-methyl-3H and L-leucine-14C into ribonucleic acid, desoxyribonucleic acid, and protein, respectively, in Ehrlich ascites tumor cells, in vitro.

(f) Absorption and Metabolism

Hwang and Schanker [83] studied the absorption of 14C-labeled carbaryl by the intestine and the lung in rats. They instilled 0.1 ml of a (0.025-0.05 mM 14C-labeled carbaryl) test solution into a tightly secured tracheal cannula (at the tracheal bifurcation) in 12 anesthetized rats. At the end of 2, 4, and 6 minutes, the lungs were removed, processed, and analyzed by liquid scintillation for the recoverable 14C-labeled material. The entire small intestine was isolated in 15 rats and 1 ml of a 0.005-0.1 millimolar 14C-labeled carbaryl aqueous solution was injected into the lumen of the small intestine. At 2, 4, 6, 8, and 10 minutes, the intestines were removed and the unabsorbed 14C label was recovered and measured by liquid scintillation. By plotting the measured amounts recovered at the various times, it was determined that the absorption half-

time for the lungs of rats was 2.6 minutes and for the intestine 6.4 minutes. By varying the known concentration of the carbaryl solution, the investigators determined that 14C-labeled carbaryl crosses the wall of the rat intestine by simple diffusion. The authors indicated that absorption through the lung was much more rapid than through the intestine and also appeared to occur by a process of simple diffusion.

Casper et al [84] examined the gastric absorption of 14C-labeled carbaryl in the isolated (empty) stomachs (with portal circulation intact) of an unspecified number of rats and found about 53% and 82% of the 14C label in the portal blood in 22 and 67 minutes, respectively. The authors verified the identity of the 14C material in the portal blood using several analytical techniques (chromatography and infrared spectroscopy) and concluded that approximately 90% of the labeled material found in the portal blood was 14C-labeled carbaryl. They concluded that carbaryl could be rapidly absorbed from the stomach in the fasting rat, but that absorption would be expected to be retarded by the presence of additional gastric contents.

In a percutaneous absorption study, Hurwood [85] studied the carbaryl residues in selected tissues (liver, kidney, muscle, and omental and perirenal fat) of steers and in the milk of cows. Fourteen steers were used in the study. Six steers were sprayed once and six were sprayed three times, at 2-day intervals, with 2 gallons of 0.3% carbaryl as a colloidal dispersion in water. Two untreated steers served as controls. Two animals from each group were killed at 1, 3, and 7 days after application of the spray for tissue analysis to detect any carbaryl residue. Perirenal fat from the steers was found to have the highest concentration in the tissues

examined 1 day after treatment. All tissue examined had some carbaryl residue 3 days after treatment. However, no detectable tissue residue was present in the steers' tissues after 7 days. Each of three dairy cows was sprayed once with 2 gallons of the 0.3% carbaryl spray, and milk samples were analyzed from both daily milkings for the next 5 days. Carbaryl was found in the greatest concentration at the first milking, 5 hours after spraying. It was excreted in the milk for at least 69 hours but was not found in milk taken 77 hours after spraying the cows.

Bukin and Filatov [86] studied the tissue distribution of carbaryl and its metabolites in rabbits. Carbaryl was administered in single oral doses ranging from 100 to 700 mg/kg. No clinical signs were observed and no carbaryl was found in the organs or tissues at autopsy, 24-80 hours after carbaryl treatment at 100, 200, or 300 mg/kg . After single doses of 400 mg/kg, an unspecified number of rabbits were killed at various intervals, and tissues, bile, and urine were analyzed for carbaryl. Residual amounts of carbaryl determined by paper chromatography are listed in Table XIII-3. At doses of 600 and 700 mg/kg, residual carbaryl ranged from 0.075 to 1.2 mg/kg in all organs and tissues examined; the time of examination was not reported however. The high dose (700 mg/kg) was fatal to all rabbits. These results suggest rapid excretion and little long-term retention of carbaryl in rabbits.

Carpenter et al, [27] in their 1961 report on the effects of carbaryl in mammals, established that approximately 31.3 ± 1.6% of the 1-naphthol portion of an orally administered dose of 0.015 g of carbaryl to each of 18 rats was recovered from their urine in 48 hours as a conjugated form, probably as the glucuronide.

In a study by Knaak et al, [34] carbaryl labeled with carbon-14 at various moieties, including the naphthyl (naphthyl-labeled), methyl (methyl-labeled) and carbonyl (carbonyl-labeled) moieties, was administered by gavage to three groups of four rats each. One group received 20.0 mg/kg each of methyl-labeled carbaryl; another group of four received 20.0 mg/kg each of naphthyl-labeled carbaryl; and the third group received 9.0 mg/kg of carbonyl-labeled carbaryl. The average total amount of labeled carbaryl which was detected and measured by a scintillation spectrometer from all sources--urine, feces, expired air (carbon dioxide), and carcasses--was 94% over a 7-day period. However, the authors stated that the excretion of carbaryl was essentially complete by the end of 3 days. Ninety-five percent of the naphthyl-labeled compound was detected in urine and feces; 99% of the carbonyl-labeled compound was found in urine, feces, and respiratory carbon dioxide; and approximately 91% of the methyl-labeled carbaryl was detected in urine, feces, carbon dioxide, and carcasses of the rats.

In the same publication, [34] the authors reported on administration of carbaryl ip to three groups of three 150-g male rats as naphthyl-labeled, methyl-labeled, or carbonyl-labeled carbaryl. Each rat was administered 3.0 mg of the appropriate labeled material in 300 mg of polyethylene glycol 400. The naphthyl- and methyl-labeled carbaryl were also administered ip at the same doses to three 200-g guinea pigs of unstated sex. From 24-hour pooled specimens of rat urine, 73, 47, and 48% of the naphthyl-, methyl- and carbonyl-labeled compounds, respectively, were recovered as carbaryl equivalents. Guinea pig urine, also 24-hour pooled specimens, yielded 85% of the total amount of naphthyl- and methyl-

labeled carbaryl administered. The urinary metabolites of the rat and guinea pig were identified from chromatographs. From those determinations, the authors showed that carbaryl, in the rat and guinea pig, is transformed into a series of eight or more metabolites. The major metabolites in this series were two glucuronides, the 4(methyl carbamoyloxy)-1-naphthyl glucuronide also known as 4-hydroxycarbaryl glucuronide and the 1-naphthyl methylimidocarbonate-O-glucuronide. Hydrolyzed products were also present; the glucuronide and the sulfate of 1-naphthol were detected in both rat and guinea pig urine after administration of the naphthyl-labeled carbaryl. The 1-naphthyl methylcarbamate N-glucuronide was present in guinea pig urine only after administration of either naphthyl- or methyl-labeled carbaryl. Table XIII-4 lists the metabolites found in rat and guinea pig urine with those of other mammals for comparison. Unidentified neutrals and other unidentified metabolites were found in excretions of both rats (one group, A) and guinea pigs (two groups, A and B). The investigators also found evidence that carbaryl could have been conjugated directly with glucuronic acid to form 1-naphthyl methylcarbamate N-glucuronide and 1-naphthyl methylimidocarbonate-O-glucuronide.

The in vitro metabolism of carbaryl by rat and guinea pig liver preparations was investigated in the same study. [34] Naphthyl-labeled carbaryl was incubated with fortified liver homogenates or microsomal preparations obtained by differential centrifugation. Metabolites formed by both rat and guinea pig liver preparations included 1-naphthyl glucuronide, 4-hydroxycarbaryl, 4-hydroxycarbaryl glucuronide, and unidentified water-soluble neutrals. The formation from carbaryl of 1-naphthyl, 1-naphthyl methylimidocarbonate-O-glucuronide, and 4-hydroxy-1-

naphthyl methylcarbamate was observed with rat, but not with guinea pig, liver preparations. These studies indicate a metabolic profile similar to that seen in the urinary excretion studies with carbaryl.

The metabolism of carbaryl in the dog was reported by Knaak and Sullivan. [87] Two forms of carbaryl, labeled with carbon-14 at either the naphthyl or methyl moiety, were separately and sequentially administered 7 days apart, in gelatin capsules to three female beagles weighing 9 kg each in doses of 25 mg/kg. Urine and feces were collected daily for 7 days and analyzed for 14C by liquid scintillation counting techniques. Although similar techniques for detection were used, those metabolites found in rats and in most other mammals were not present in dog urine (Table XIII-4). The beagles did not seem to be able to excrete unconjugated 1-naphthol or hydroxylate carbaryl. However, it probably can form naphthyl glucuronide and sulfate, and it does seem to be able to conjugate carbaryl directly. The only other metabolite found in dog urine was 1-naphthyl methylimidocarbonate-o-glucuronide. The dog also differs from the rat in the route and quantity of excretion. Almost half the total carbaryl equivalents excreted by the dog were found in the feces and were accounted for by the naphthyl label, whereas only about 10% were found in the rat feces. [34] Rat urine yielded about 68% [34] and dog urine about 23% [87] of the dose as the methyl label.

Knaak et al, [33] using techniques similar to those they used in studies of other mammals, [34,87] examined fecal and urinary metabolites in two young female swine weighing 14.5 and 18 kg. [33] One animal was given methyl-labeled carbaryl and the other naphthyl-labeled carbaryl at doses of 25 mg/kg in gelatin capsules. Urine and feces were collected over a 5-day

period. The pigs excreted 83.4 and 1.6% of the naphthyl label in urine and feces, respectively, and 70 and 1.0% of the methyl label in urine and feces, respectively. Two major metabolites, 1-naphthyl methylimidocarbonate-o-glucuronide and 4(methylcarbamoyloxy)-1-naphthyl glucuronide, were identified. In addition, several unidentified metabolites and 1-naphthyl glucuronide from the naphthyl label were obtained. Swine metabolites are listed with others in Table XIII-4. In the same report, [33] the metabolism of carbaryl in sheep was described. One 42-kg ewe was given a dose of 25 mg/kg of naphthyl-labeled carbaryl orally in gelatin capsules; 2 weeks later a similar procedure was used to administer methyl-labeled carbaryl. Three major metabolites excreted were 1-naphthyl methylimidocarbonate-o-glucuronide, 4(methylcarbamoyloxy)-1-naphthyl glucuronide, and 1-naphthyl sulfate. The two metabolites having only the naphthyl label were 1-naphthyl glucuronide and the sulfate. The sheep metabolites are listed with others in Table XIII-4.

One female rhesus monkey weighing 4.6 kg was orally administered labeled carbaryl at a dose of 300 mg/kg, in two phases. [33] In the first phase, the naphthyl-labeled form was ingested, and, in the second phase 4 days later, the methyl-labeled form of carbaryl was administered. The monkey excreted in the urine two major metabolites: 1-naphthyl methylimidocarbonate-o-glucuronide and 4(methylcarbamoyloxy)-1-naphthyl glucuronide. Small quantities of the sulfate-conjugated naphthyl and 4-hydroxycarbaryl were excreted in monkey urine. The monkey metabolites are listed in Table XIII-4.

In Effects on Humans, the results of studies by Knaak et al [33] on the urinary metabolites of carbaryl in two men were discussed. The men,

weighing approximately 81 and 86 kg, ingested carbaryl in gelatin capsules at doses of 2 mg/kg. Urine was collected before administration, for control determinations, and for 4 days after administration. Only 26-27% of the dose was accounted for in the composite 4-day urine collected from the two men. The following were identified from the urine specimens: 1-naphthyl glucuronide and sulfate, and conjugates of glucuronic and sulfuric acids, 4-(methylcarbamoyloxy)-1-naphthyl glucuronide. The human metabolites are presented in Table XIII-4 for comparison with six other mammals.

In view of the possible relevance of the metabolism of carbaryl with respect to its teratogenic potential in various mammalian species, it is important to identify those species that metabolize the compound similarly or dissimilarly to humans. The results of the preceding studies on the metabolism of carbaryl in human beings, rats, guinea pigs, sheep, monkeys, swine, and dogs [33,34,87] show that the similarity of metabolic products allows these species, with the exception of the dog, to be divided into two groups: in the first, humans, rats, guinea pigs, and sheep, and in the second, monkeys and swine. Low doses of carbaryl were reported [66] to cause teratogenic effects in the beagle dog. (See previous discussion of reproduction studies.) However, unlike humans, the beagle does not excrete 1-naphthol nor does it hydroxylate carbaryl. Carbaryl was not found to be a teratogen in monkeys [67,69] and rats, [70] but in one study carbaryl produced terata in guinea pigs at very high doses, [71] while in another study of the same species using moderately high doses carbaryl was not found to be teratogenic. [72] Present studies show that the metabolism of carbaryl in the dog differs from that in humans, monkeys, rats, and guinea

pigs, so it is unwarranted now to extrapolate from dogs to humans regarding the teratogenic potential of carbaryl.

The excreted metabolites identified in rats, humans, and guinea pigs [34]; in monkeys, swine, sheep, and humans [33]; and in the dog [87] have been summarized in Table XIII-4 so that the metabolic pathways of carbaryl in several species may be compared.

Correlation of Exposure and Effect

Carbaryl, a methyl carbamate compound, has anticholinesterase properties. [5] Therefore, regardless of its route of entry, once absorbed, it can induce signs and symptoms of toxicity by causing an increase of acetylcholine at its sites of action in the central, autonomic, and peripheral nervous systems. This increase in acetylcholine is a consequence of the inhibition of acetylcholinesterase, the enzyme which hydrolyzes acetylcholine. [2,5] The relationship of the inhibition of cholinesterase in plasma and erythrocytes to the inhibition of acetylcholine in the nervous system and at other sites of action has not been clearly established.

Evaluation of the available information suggests that carbaryl may be absorbed after oral ingestion, [40,41] during exposure to airborne concentrations, [28] or upon direct dermal contact. [30,31] The signs and symptoms observed as a consequence of exposure to carbaryl in the workplace are clearly manifestations of excessive cholinergic stimulation, and thus inhalation or dermal exposure resulted in the following signs and symptoms: nausea and dizziness, headache, perspiration [13 (sec 7)]; headache, nausea, vomiting, mild abdominal cramping, dimness of vision [13 (sec 13)];

skin rash [35]; and weakness, dizziness, and difficulty in breathing. [38] Oral ingestion, usually unrelated to occupational exposure, has resulted in the following signs and symptoms, most of which are also due to excessive cholinergic stimulation: miosis, excessive salivation, and incoordination [28]; epigastric pain, sweating [21]; disturbed vision, and pulmonary edema [40]; nausea, vomiting, hyperreflexia, pallor, intestinal colic, nasal discharge, salivation, headache, lacrimation, and tremors [41]; difficulty in sleeping, and abdominal cramping. [32] Overexposure to carbaryl in the workplace environment apparently results in a rapid onset of symptoms which causes voluntary cessation of work and termination of exposure. [13 (sec 13),20] In addition, employees who have been overexposed to carbaryl in the workplace apparently recover rapidly. [13 (sec 7,13)]

Animal studies have indicated that absorption of carbaryl by the lungs, [83] intestine, [83] and stomach [84] is fairly rapid. The characteristics of the urinary metabolites encountered in both humans [33,34] and animal species [33,34,87] would indicate that the liver is the primary site for metabolizing carbaryl after its absorption by various routes. Support for this hypothesis is found from in vitro studies [34] using carbaryl incubated with rat and guinea pig liver preparations which resulted in the formation of metabolites almost identical to those observed in the urine of animals treated with carbaryl.

Several studies by Knaak and coworkers [33,34,87] compared metabolic processes of carbaryl in seven mammals, namely rat, guinea pig, dog, swine, sheep, monkey, and human. Table XIII-4 lists the various metabolites and the means by which they were detected. It was concluded [87] that the dog (beagle) probably conjugates carbaryl and excretes much of the degradation

products in the feces, unlike most of the other species, in which the urine is the major excretory route. Most of the other mammals hydrolyze and hydroxylate, as well as conjugate, carbaryl before excretion. From the studies of Knaak and coworkers, [33,34,87] humans, rats, guinea pigs, and sheep metabolize carbaryl in one manner, monkeys and swine in another, and dogs in a third. One metabolite which appeared in the urine of all species, except the dog, was conjugated 1-naphthol [33,34,87] which might be useful in assessing carbaryl exposure. [13 (sec 7,9,10),28,32-35]

Best and Murray [28] observed 59 employees during a 19-month period in a carbaryl-manufacturing plant where airborne concentrations of carbaryl ranged from 0.03 to 40 mg/cu m. Their report indicated that relatively large quantities of 1-naphthol were excreted in urine, that blood cholinesterase activity was either within the normal range or slightly inhibited, and that at no time did any of the employees studied have clinical or subjective evidence of increased acetylcholine activity. However, the methods used to determine cholinesterase activity in this study probably underestimated the degree of cholinesterase inhibition.

Williams [13 (sec 10)] reported only limited data suggesting that airborne concentrations of carbaryl around 50 mg/cu m during an approximate 8-hour workday did not produce symptoms of toxicity if the workers were provided either respiratory or dermal protective equipment. The report of Yakim [39] indicated that reductions in blood cholinesterase activity occurred following 4- to 6-hour exposures of workers for 3-4 days to an average airborne carbaryl concentration of 2 or 4 mg/cu m. He did not adequately describe how he measured the airborne concentrations or the method used to determine cholinesterase activity. At these concentrations

and durations of exposure, no symptomatic changes due to anticholinesterase activity were reported by the author. It is apparent from the literature reviewed that the airborne carbaryl concentration at which significant signs and symptoms may first appear in humans has yet to be determined. Vandekar [35] observed no adverse effects when sprayers and 95 villagers in Nigeria were exposed to carbaryl from an application of a 5% solution in their dwellings.

Several inhalation studies [27,39] on animals exposed to airborne carbaryl have been conducted. Carbaryl at a concentration of 390 mg/cu m has been shown to produce nasal and ocular irritation in guinea pigs upon 4-hour exposure. [27] Exposure concentrations of 63 mg/cu m in cats [39] and 75 mg/cu m in dogs [27] produced signs of cholinesterase inhibition within 2-5 hours of exposure. Rats exposed to carbaryl dust in air at an average concentration of 10 mg/cu m (5-20 mg/cu m) for 7 hours/day, 5 days/week, for 90 days did not show any grossly visible changes, and all the animals survived. [27] Microscopic examination of several tissues including lung taken from these animals revealed no carbaryl-associated lesions. Yakim [39] exposed four cats at an airborne carbaryl concentration of 63 mg/cu m for 6 hours/day for a month. Cholinergic stimulation, indicated by periodic salivation, was observed during the first 2 hours of exposure each day. Exposure at an average concentration of 40 mg/cu m for 6 hours/day for 2 months produced some deterioration in conditioned reflexes of the cats. No further information was given. No signs of toxicity were observed upon exposure of cats for 4 months (6 hours/day) at a concentration of 16 mg/cu m, while at 40 mg/cu m erythrocyte cholinesterase activity dropped to 50% or less. The method for

determining cholinesterase activity was not disclosed. [39] The above studies [27,39] indicate that typical signs of cholinergic stimulation are evident at airborne concentrations of 63 and 75 mg/cu m in the cat and dog, respectively, while exposure at 16 mg/cu m appears to constitute a no-effect level with respect to toxic manifestations of cholinesterase inhibition in the cat. [39]

Dermal absorption of carbaryl has been investigated in both humans and experimental animals. In two dermal absorption studies on humans, [30,31] approximately 74 and 70% of 14C-labeled carbaryl were recovered from urine within 5 days after application of carbaryl in acetone to the skin of the forearm and of the face near the angle of the jaw, respectively. In another study [13 (sec 9,10)] comparing absorption after inhalation and dermal exposure in humans, two employees in a plant manufacturing carbaryl were exposed at approximately the same airborne concentrations on each of 2 days. Employee A was protected from skin contact but not from inhalation of carbaryl; employee B was protected from airborne carbaryl but not from carbaryl contact on his arms and hands. Measurement of 1-naphthol (a carbaryl metabolite) in control and postexposure urine revealed that the concentration of urinary 1-naphthol excreted by employee A was over 3,600 and 2,400 μg/100 ml and by employee B over 7,000 and more than 500 μg/100 ml, on days 1 and 2, respectively, thus indicating that, under similar exposures, lungs and skin readily absorb carbaryl. No topical reactions have been reported in humans except for a single case [35] of a rash of uncertain cause associated with carbaryl exposure, and no dermal irritation has been reported in carbaryl-treated experimental animals when carbaryl was directly applied. [27,39] A slight

degree of local eye injury and necrosis has been found in rabbits when carbaryl was applied directly to the eye, and guinea pigs were weakly sensitized in a skin-sensitivity test. [27] In addition, miosis and conjunctival hyperemia, which are signs of anticholinesterase activity, also have been observed in rabbits' eyes treated locally. [39] In cattle sprayed with carbaryl, the compound was present in all body tissues examined 1 and 3 days, but not 7 days, after exposure. [85] The concentration of carbaryl in the milk of cows was highest 5 hours after skin exposure but fell to an undetectable level 77 hours after spray application. No detectable carbaryl residue was found in meat of steers 7 days after spraying.

A few studies on the effects of carbaryl administered orally to humans have been reported. A report [40] of one case of suicide attributed to carbaryl has been found. This incident is clouded by the fact that the subject was treated with an oxime (PAM), which is usually recommended for treatment of poisonings due to excessive absorption of organophosphate anticholinesterase agents, but which has had an adverse effect on animals poisoned with carbaryl. [45,46]

A single oral dose of 250 mg of carbaryl (approximately 2.8 mg/kg) produced early symptoms including sweating and epigastric pain in an adult male [21] who intentionally swallowed the chemical. [29] A rapid recovery followed treatment with atropine. [21] Best and Murray [28] described a case in which an unknown amount of carbaryl was ingested by a 19-month-old infant who then had typical signs of anticholinesterase intoxication including miosis, excessive salivation, and incoordination. Recovery was complete after atropine treatment. Lopez [41] described light intoxication

in a young man who ate watermelon which had been sprayed with 80% carbaryl, and in another person who drank several milliliters of a solution of 80% carbaryl. Symptoms reported by the author in the men were nausea, vomiting, hyperreflexia, pallor, intestinal colic, nasal discharge, salivation, headache, lacrimation, and tremors.

Daily oral carbaryl doses of 0.12 mg/kg in gelatin capsules administered to 6 volunteers for 6 weeks caused no changes except an apparently slight decrease in the ability of the proximal convoluted tubules of the kidney to reabsorb amino acids as assessed by the urinary amino acid nitrogen to creatinine ratio. [32] This functional, but reversible, alteration was reported to be present only in the group of volunteers who ingested 0.12 mg/kg/day, and not in the group receiving 0.06 mg/kg/day, in which the urinary amino acid nitrogen to creatine ratios were lower than the controls. Repeated oral administration of carbaryl produced mild renal changes in rhesus monkeys, [44,51] rats, and dogs. [27] The oral doses administered to experimental animals were considerably higher, viz, 600 mg/kg in the monkey at an unstated length and frequency, 7.2 mg/kg in the dog for 1 year, and 400 ppm in the diet of rats for 2 years, than the dose required to produce the changes in human renal function reported by Wills et al. [32] Furthermore, the renal changes in rats were not significant after 2 years of administration; in the dogs, the same cloudy swelling of the renal tubules was found in the experimental and, to a lesser extent, in the control groups. [27] Doses of 0.12 mg/kg of carbaryl administered to man were reported by the authors to be responsible for reversible renal tubular dysfunction. [32] Since calculation of the ratios was carried out on only 5 subjects and that differences apparent from the

graphic illustration of the data are not striking, the results presented in this study are difficult to interpret. As discussed earlier, it is difficult to draw conclusions from the vacuoles seen in a single electron photomicrograph of the monkey kidney tissue studies. [44,51] The long-term administration of carbaryl to rats and dogs [27] resulted in some cases in an incidence of renal damage only slightly greater in test animals than in the controls. Considering all the present data, the evidence for renal dysfunction is at best suggestive but not conclusive.

In order to study the paralytic effects of carbaryl, Carpenter et al [27] administered the compound at very high doses to chickens (0.25-3 g/kg). There was leg weakness at doses greater than 1 g/kg, attributed by the authors to transient cholinergic effects, but no microscopic evidence of demyelination. Another study in chickens by Gaines [43] lends support to the conclusion of Carpenter et al. [27] Smalley et al [52] showed that repeated administration of large doses of carbaryl to swine (150-300 mg/kg) produced ataxia and prostration without demyelination, and, in another study [55] at a dose of 125 mg/kg, the compound failed to produce paralysis in dogs but did produce paraplegia and spastic paresis in miniature pigs. [55] Carbaryl did not cause any demyelination in the chicken, the animal of choice for detecting this form of paralysis, and, since no data have been found concerning demyelinating paralytic effects of carbaryl in humans, it is concluded that, based on available evidence, carbaryl is not likely to cause chronic neurotoxicity, ie, Ginger Jake paralysis.

The effects of carbaryl on the developing fetus have been investigated in several studies. [66,67,69-71] Smalley et al [66] described reduced viability of pups and dystocia, probably maternal, in

beagle dams given oral doses of carbaryl ranging from 3.25 to 50 mg/kg throughout gestation. Litter size was reduced as compared to controls in the 25 and 50 mg/kg dose groups. Teratogenic effects were seen at doses ranging from 6.25 to 50 mg/kg. In a study [67] on primates, the abortion rate was increased at carbaryl doses as low as 2 mg/kg given orally. This effect was not dose related, since a dose of 20 mg/kg produced only half as many abortions (3 out of 6) as the 2 mg/kg dose (2 of 2). In addition, the small number of pregnant animals in each group (2 and 6, respectively) and in the control group (5 animals) prevents reliable conclusions based on a statistical evaluation. [67] A later report by Dougherty and Coulston [69] describes another monkey study using 79 animals. These investigators observed no signs of toxicity in adult females, no increase in abortions over controls, and no fetal abnormalities in monkeys given carbaryl (range 0.2-20 mg/kg/day) during days 20-38 of gestation.

Robens [71] found that fetal deaths and terata occurred in guinea pigs administered carbaryl at a dose of 300 mg/kg during various intervals of gestation. This dose was also lethal to some dams, and the author indicated that this dose, which produced terata and maternal deaths in guinea pigs, was at least 1,000 times the level of carbaryl allowed in human food. The high mortality rate in the pregnant dams at this high dosage makes a conclusion of teratogenicity in this study difficult since another study by Weil et al [72] in the same species reported no carbaryl-related teratogenicity at dietary and intubation levels of 300 and 200 mg/kg, respectively. Doses of 50, 100, and 200 mg/kg of carbaryl given to pregnant rabbits [71] produced no adverse effects on dams or offspring. In hamsters [71] given carbaryl at doses of 125 and 250 mg/kg during

gestation, fetal mortality was higher than in controls, but no terata were found. Rats were administered carbaryl in the diet at doses of 20, 100, and 500 mg/kg at various intervals throughout pregnancy or until weaning of the pups. [70] No teratogenic effects that could be attributed to carbaryl were observed at any dose level. As discussed earlier, the dog differs markedly from several other species, including humans, in its metabolism of carbaryl. This difference could account for the teratogenic effects observed in the study by Smalley et al. [66] The lack of carbaryl-related teratogenicity in other species does not now warrant the conclusion that carbaryl should be classified as a teratogen in humans.

Effects of carbaryl on various aspects of the reproductive cycle of rodents have been observed at doses given either in the diet or by oral intubation, ranging from 2 to 20 mg/kg and from 2,000 to 10,000 ppm. [72,73,78] Effects seen in rats with doses as low as 2 and 5 mg/kg included decreased spermatogenesis, sperm motility, and duration of estrus. [73] Decreased litter size and reduced survival of the pups have been observed with varying frequencies in rats and gerbils at all doses studied [73,78] except in a three-generation reproduction study of rats conducted by Weil et al [72] in which adverse effects on viability of pups and litter size, as well as decreased fertility and lengthened gestation period, were seen only at doses of 100 mg/kg given by oral intubation, and not at lower doses. In the same study, [72] lengthened gestation periods in rats administered carbaryl in the diet were seen only at a dose of 200 mg/kg. Collins et al [78] studied rat parents and offspring in a three-generation reproduction study. Doses of 2,000, 5,000, and 10,000 ppm carbaryl in the diet showed that, at the highest dose and to a lesser extent at the next

lower dose, the viability of the pups, survival of offspring, and litter size decreased from the first generation on. The lowest dose (2,000 ppm) affected only body weight gain of the parents and weanlings. In a similar three-generation reproduction study on gerbils given carbaryl in the diet (2,000, 4,000, 6,000, and 10,000 ppm), Collins et al [78] reported that no litters were produced in the F3b generation at 10,000 ppm, and that there was a decrease in fertility, pup viability, litter size, and survival of pups from day 4 to weaning which appeared sporadically at all doses. Collins et al [78] suggested that the effect of carbaryl on reproduction was due to an indirect effect on the testes and ova mediated through the hypothalamohypophyseal complex. Carbaryl given to rats by gastric intubation at doses of 2 and 5 mg/kg produced adverse effects on fertility and survival of the pups during the first month of life. [73] The same doses produced a decrease in spermatogenesis and a decreased duration of estrus. In addition, microscopic changes of an adverse nature were noted in ovarian follicles and spermiogenic epithelium of the testes after carbaryl treatment.

In another study, Shtenberg and Rybakova [77] administered carbaryl orally to rats of both sexes at doses of 7, 14, and 70 mg/kg for up to 12 months. Growth of rats was inhibited at 14 and 70 mg/kg, and sperm motility in males also decreased in a dose-related manner after 12 months of treatment. Degenerative changes in the testes, including edema of interstitial tissue and destruction of germinal epithelium, were noted in carbaryl-treated male rats. In females, the estrus cycle was prolonged at the 14 and 70 mg/kg doses. From the above studies, [72,73,77,78] it may be concluded that oral administration of carbaryl to rodents has an effect on

several aspects of their reproduction. However, it is difficult to relate these effects from oral administration at high doses to rodents to those encountered by inhalation and dermal absorption in humans, at lower doses in the workplace environment.

From the results of a screening experiment for dominant-lethal mutagenic effects, [59] in which mice received carbaryl at doses up to 1,000 mg/kg for 5 days, there is no evidence that carbaryl is a dominant-lethal mutagen in mice. More recent studies on bacteria [63,65] and on yeast [64] indicate that carbaryl is not a mutagen; however, experiments on an insect indicates that it is a weak mutagen. [62] Nitrosocarbaryl in several microbiologic studies [63-65] proved to be a strong mutagen. The significance of this finding in terms of its relevance to humans exposed to carbaryl remains to be determined.

Shimkin et al [81] synthesized N-methyl naphthylcarbamate and compared it in mice to 21 other carbamates using a pulmonary tumor bioassay. While some of the carbamates were positive, the synthesized compound was classed as marginal. Shimkin et al [81] classified the synthesized N-methyl naphthylcarbamate as a marginal tumorigen in mice; however, the lack of information on the purity of the compound and the structural formula written in the study leave doubt as to whether 2-naphthyl or 1-naphthyl N-methyl carbamate (carbaryl) was tested and make the results of the study difficult, if not impossible, to interpret.

Oral administration of carbaryl to mice (4.6 mg/kg/day for 18 months) revealed no significant evidence of tumorigenicity. [79] Subcutaneous injection of carbaryl, approximately 400 mg/kg/week, into tumor-susceptible mice for 5 months did not increase the incidence of tumors over that in the

control animals. [27] No dose-related incidence of tumors or increase in tumors over the controls was established by Carpenter et al [27] in rats given carbaryl in the diet at levels of 50, 100, 200, and 400 ppm for a period of 2 years. Andrianova and Alekseyev [80] found tumors in mongrel rats given carbaryl orally (30 mg/kg twice weekly) or by subcutaneous implantation (paraffin capsules containing 20 mg of carbaryl) for 22 months. The results of this study are not conclusive because of the high mortality in the experimental group, the absence of sufficient control and other background data, and the fact that the carbaryl used in these experiments was probably contaminated. An evaluation of the carcinogenic studies [27,79-81] does not now warrant a conclusion that carbaryl is a carcinogen.