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CARBARYL

A Toxicological Review and Risk Analysis

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INTRODUCTION

Carbaryl (1-naphthyl-N-methylcarbamate) is the most widely used carbamate anticholinesterase insecticide and is marketed as SEVIN® brand carbaryl, a Union Carbide product. The chemical structure of carbaryl is shown in Fig. 1. Since the 1960s carbamate insecticides have steadily replaced the more persistent chlorinated hydrocarbon products.

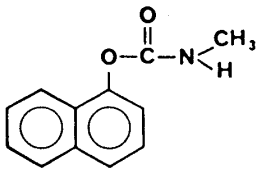


FIG. 1. Chemical structure of carbaryl (1-naphtyl-N-methylcarbamate); molecular weight: 201.2.

The preparation of a monograph describing the toxicological literature relevant to understanding the key issues impacting on the regulation of carbaryl was first considered by this reviewer because of the widespread interest in the US EPA Health Effects Document of 1980. This need was partially satisfied by the excellent review of Mount and Ohme (1982). A very selective and noncomprehensive synopsis of Russian literature was prepared by the United Nations Environment Program (UNEP,1982). The shortcomings of the UNEP report are especially important because, while previous reviews of carbaryl had provided excellent analysis of the English language publications, the coverage of the Russian literature was constrained by lack of availability. Another limitation of earlier reviews was the lack of availability of unpublished data known to regulatory agencies and Union Carbide.

Union Carbide was requested to make available for examination all toxicological data known to the corporation. Union Carbide agreed and documents totaling over 40,000 pages were considered. Seventy-five complete translations of Russian research papers and several hundred Russian abstracts were also provided by Union Carbide.

This monograph considers the potential of experimental systems exposed to carbaryl to express: (I) neurotoxicity, (II) developmental toxicity, (III) mutagenicity, (IV) oncogenicity, (V) immunotoxicity and viral enhancement. Human exposure (VI) and an analysis of potential to present a cancer risk to humans (VII) is also discussed. N-nitrosocarbaryl, a

nitrosamide reaction product of carbaryl, is integrated into the sections on mutagenicity, oncogenicity, human exposure and risk.

Reports are described in the context of the total pertinent literature. Emphasis has been placed on selected Russian studies in the discussions of neurotoxicology, developmental toxicology, mutagenesis and oncology, in an effort to facilitate cross reference to the Russian review offered by UNEP and to address issues raised by these publications. Comments on the completeness of specific reports, statistical analyses, or authors' conclusions are offered only when uncertainty or controversy exists or the reader is encouraged to study the original article. Articles will be discussed in chronological order under each topic heading.

The international, interdisciplinary journal *NeuroToxicology™* was selected for publication of this review since the journal's policy is to "publish reviews which critically analyze topics of current interest in neurotoxicology" and "papers dealing with the effects of neurotoxicants on other systems (e.g., reproductive, immune)".

SUMMARY AND OVERVIEW

Neurotoxicity

Neurotoxicity is defined as impairment of structure or function of the nervous system. Great differences exist in neurotoxic sensitivity to carbaryl. Carbaryl is very potent as an anticholinesterase poison when its use is directed against target insect species. Non-target vertebrates species are among the most resistant.

Forced oral exposures (in excess of 200 mg/kg) of carbaryl are required to kill mammals. Death requires the inhibition of a major portion of the cholinesterase of the central nervous system (CNS).

Sub-lethal doses of carbaryl (10-100 mg/kg) can produce a profound but rapidly reversible effect on the neurological function of a variety of vertebrate species. Delayed and irreversible neurotoxic effects have not been observed in vertebrate species including man. Reversible short-term neurotoxic effects have been observed in every species tested but only at levels of exposure producing overt clinical signs of intoxication. Dietary exposure presents no risk of neurotoxicity.

Developmental Toxicity

Developmental toxicity is defined as any adverse effect on reproductive performance. The potential of carbaryl to produce developmental toxicity has been studied in numerous mammalian species including the rat, mouse, gerbil, hamster, rabbit, swine, dog and primate, utilizing a wide variety of study designs and routes of administration. Frank terata (structural malformations) have been produced in the guinea pig, rabbit, and dog, only at exposure levels obviously toxic to the pregnant animal. The monkey has yielded negative results. Doses tested often approach the LD_{50}. Risk of developmental toxicity resulting from dietary exposure to carbaryl in humans is likely to be vanishingly small since all observed effects required exposure levels which produced significant cholinesterase inhibition and commonly other obvious cholinergic clinical signs of overt poisoning.

Mutagenicity

Mutagenicity is defined as the process of permanently altering the genetic complement of an entity capable of reproduction, in such a way as to permit propogation

of an altered genome. At high doses, usually toxic to the test system, carbaryl can produce changes in or disruption of the target genetic apparatus of several predictive screening tests.

Nitrite ions can react under acidic conditions with amines, amides or ureas to produce N-nitrosamines or N-nitrosamides. N-nitrosamide is a nitrosamine reaction product of nitrite and carbaryl and is highly mutagenic in some *in vitro* systems.

Although numerous tests have been conducted, no data from an *in vivo* mammalian system supports the contention that carbaryl usage poses a hazard to future generations of mammals via the induction of modifications in the heritable genic complement of either germinal or somatic cells.

Oncogenicity

Oncogenicity is defined as the ability of a substance to induce neoplasia in living animals. A number of longterm oncogenicity-carcinogenicity studies have been conducted with carbaryl. These studies have utilized rats, mice and dogs. No oncogenicity has been attributed to carbaryl in any of these studies. Carbaryl when co-administered with background nitrite has not been shown to induce or promote cancer. N-nitrosocarbaryl, although carcinogenic when administered at high doses, does not represent a significant risk factor.

Immunotoxicity

Immunotoxicity is defined as an effect which alters or impedes the immune system's ability to perform its normal function. Carbaryl, when administered at doses producing subclinical neurotoxic symptoms has been reported to produce a variety of effects on the immune system which are reversible and non life-threatening. Lifetime exposure to carbaryl has not resulted in increased infectious disease or cancer in rats and mice. Most studies in rabbits, mice and rats, at doses permitting survival, have not produced significant effects on the immune system. Carbaryl does not appear to represent a risk factor to the human immune system.

Viral enhancement is defined as the ability to modify the biological behavior of a virus or its host in such a way that proliferation and or virulence is increased. Enhancement may result from an action directly on the virus, as an indirect effect on the immune system or combinations of both. Epidemiologic studies have not linked carbaryl exposures with Reyes' Syndrome or any other viral disease. A number of xenobiotics, including carbaryl, have been shown, in tissue culture experiments, to be capable of enhancing viral growth. Extrapolating from *in vitro* viral enhancement experiments in tissue culture to *in vivo* experiences and expectations in humans presents problems which are currently unresolved.

Human Exposure

Human exposure to carbaryl via respiratory and dermal routes has been studied in individuals involved in the manufacture, formulation, packaging and, distribution and application of carbaryl for some 25 years of the product's commercial life. Other studies have provided data on the toxic and nontoxic effects of carbaryl following oral ingestion by humans and have provided insight into metabolic pathways. Despite general availability, high volume and diverse use, reports on incidents of intoxication with carbaryl, even including suicide attempts, indicate very rare incidence of mortality. Epidemiological studies have not demonstrated delayed neurotoxic effects, dysmorphic sperm or viral enhancement in humans exposed to carbaryl.

Risk Analysis

The possibility of cancer risks due to carbaryl exposures are limited to concern for N-nitrosocarbaryl. Even when highly speculative theoretical exposures to N-nitrosocarbaryl are evaluated the risks calculated are vanishingly small and well below the accepted level of concern to regulatory agencies.

I: NEUROTOXICITY

Summary

Carbaryl is a primary neurotoxicant. Sections II, III, IV, and V describe other possible effects attributable to carbaryl. Most, possibly all, of those effects are acheivable only at levels also producing detectable neurotoxicity.

Acute signs of neurotoxicity due to exposure to large quantities of carbaryl are typical of anticholinesterase carbamates and include muscarinic, nicotinic, and central nervous system (CNS) cholinergic effects. Muscarinic symptoms include salivation, respiratory secretions and bronchial constriction resulting in dyspnea and pupillary constriction. Colic, diarrhea, and frequent urination are also muscarinic effects. Nicotinic symptoms include muscle fasiculations which progress in severity to body tremors and paralysis. Respiratory muscle paralysis also occurs as a result of nicotinic action. Muscular weakness and collapse are nicotinic effects and follow the initial muscarinic signs. Bradycardia results from muscarinic action, but this may be overridden by the nicotinic action and result in tachycardia. Depression and respiratory paralysis are associated with CNS effects, and convulsive seizures may occur. Death can occur from respiratory interference associated with bronchial constriction, excessive pulmonary secretions, and respiratory depression due to paralysis of the respiratory center and/or the respiratory muscles.

The clinical course of carbaryl intoxication in animals has been described by Boyd and Boulanger (1968) for rats; for pigs by Smally *et al.* (1969); and by Carpenter *et al.* (1961) for dogs. The clinical picture of severe carbaryl intoxication is similar for mammalian species. The first signs to be noticed 15 to 30 minutes after oral administration are salivation and increased respiration followed by lacrimation, urination, defecation, and muscular twitching. Tremors can progress to mild convulsions after about 90 minutes. Constriction of pupils, profuse salivation, poor coordination, further increase in respiratory rate, and loss of bowel and bladder control are often observed in severe cases by 3 hours. These symptoms are followed in the most intensive intoxications by violent intestinal movements associated with weakness and muscular spasms. General agitation will usually subside in survivors after 5 to 6 hours. Lacrimation, salivation, slight constriction of pupils, poor coordination, muscular twitching will remain but at a progressively reduced level. Pupils, salivation and coordination of survivors will be near normal by 6-10 hours. Minimal clinical signs of peripheral nervous system or central nervous sustem symptoms can remain 24 hr after dosing. The clinical course for humans is described in VI: Human Exposure.

Administration of a sub-lethal dose of carbaryl will produce a profound but transitory effect on the neurologic function of a variety of vertebrate species. These effects are usually manifested as impaired ability to perform complex tasks, often with simultaneous clinical symptoms of cholinergic stimulation. No acceptably documented reports of persistent neurologic dysfunction following cessation of carbaryl administration were found during the preparation of this monograph. Repeated administration of high doses

of carbaryl may occasionally result in histological changes (Azizova, 1976) and even paralysis in swine (Miller *et al.*, 1973). The administration of up to 3,000 mg/kg of carbaryl to chickens produced no persistent or delayed neurotoxic effects. There is no evidence that persistent or delayed neurotoxicological changes, similar to those induced by a number of organophosphate esters, have occurred in humans due to exposure to carbaryl at any level.

Mechanism of Action

Carbaryl is toxic because of its effects on the nervous system and the subsequent impaired function of controlled systems. The nervous system is very sensitive and even transient dysfunction can result in irreversible damage. The lethal effects of carbaryl can be produced by either primary or a combination of primary and secondary effects. The major secondary effect is respiratory collapse and subsequent reduction of oxygen to the brain.

Carbaryl can act directly on special parts of the nervous system at high enough doses. The term central nervous system (CNS) refers to the brain and spinal cord of mammals and is the integrating and determining component. The peripheral nervous system (PNS) consists of afferent nerves bringing information to the CNS and efferent nerves taking instructions to muscles and glands. Nerve impulse transmission is axonic when an impulse travels along the axon and synaptic at the synapse where a burst of transmitter chemical is released. The transmitter diffuses across the synapse and completes the message at the receptor. Acetylcholine is one kind of transmitter. In order to restore the sensitivity of the receptor to new packets of transmitter, the acetylcholine at the receptor must be continually eliminated. Acetylcholinesterase is the enzyme of importance in this discussion since carbaryl is an acetylcholinesterase inhibitor and synaptic toxin.

The autonomic nerves are all efferent and carry impulses to glands and also to the muscles which are not voluntarily controlled, such as those of the intestine and the pupil. The peripheral nerves of the parasympathetic system are all cholinergic as are the adrenal medulla and sweat glands of the sympathetic system.

Autonomic nerves have synapses in the CNS. Autonomic nerves always have two peripheral synapses, the neuroeffector junction of the muscle or gland, and the intermediate. The intermediate synapse of both the sympathetic and parasympathetic systems are cholinergic.

Not all cholinergic junctions are identical. The neuromuscular junction and the parasympathetic ganglia are stimulated by nicotine and the symptoms which are produced when they are stimulated include effects on voluntary muscles, such as paralysis and fasciculations. The parasympathetic neuroeffector junctions are stimulated by muscarine. Muscarinic symptoms include slowing of the heart, constriction of the pupils, urination and salivation.

The cholinergic junctions can be classified into four categories for convenience of reference on the basis of their sensitivity to drugs. One class contains only the skeletal neuromuscular junctions, where nerve and voluntary muscle meet. Such junctions are affected by nicotine and blocked by curare, but not by atropine. When the neuromuscular junctions are blocked the muscle is paralyzed. When junctions are overstimulated fasciculation occurs.

Another cholinergic junction, important in producing common effects observed in overt carbaryl poisoning, is the neuroeffector junctions of the parasympathetic system. These junctions are located where the parasympathetic nerves meet the muscles and glands. They innervate and include the iris of the eye, the bladder, the heart, tear glands, salivary glands. The neuroeffector junctions of the parasympathetic system are not affected by nicotine or curare, but they are blocked by atropine. Muscarine is a neuropharmacologic drug obtained from certain poisonous mushrooms. Neurological

stimulation due to muscarine administration are referred to as "muscarinic effects," and include constriction of the pupil (myosis), urination, tearing, and salivation. Many of the early symptoms of carbaryl poisoning are muscarinic can be reversed or attenuated by atropine.

Only of modest importance to carbaryl poisoning are the cholinergeric junctions of the sympathetic and parasympathetic autonomic ganglia. These junctions are cholinergic, therefore acetylcholine can stimulate the sympathetic and parasympathetic systems. In addition they are affected by nicotine but not by muscarine, atropine, or curare, except at high concentrations. Sympathetic nerves innervate many smooth muscles of the eye, the bladder, the heart, and salivary glands. Parasympathetic and sympathetic nerves can operate antagonistically; thus the former slows the heart and constricts the pupil while the latter accelerates the heart and dilates the pupil. The effects of ganglionic drugs are hard to predict since they often depend on whether the sympathetic or parasympathetic ganglion is the more affected. The parasympathetic ganglion controls the muscular activity of the bladder. The sympathetic ganglion controls the blood supply.

The final group, which is important in carbaryl poisoning, are the cholinergic junctions of the central nervous system. The respiratory center of the brain is cholinergic. The respiratory center controls respiration rate and breathing stops if the center is blocked. Presumably convulsions are mediated via central neurons since convulsion are more coordinated than fasciculation, and could reasonably be expected to originate centrally. At least some CNS junctions are affected by nicotine, while others, particularly those of the respiratory center, by atropine. Ionic compounds have virtually no effects on CNS junctions, and injected acetylcholine, muscarine, or curare have little effect. Nicotine can produce convulsions, however the term nicotinic effect is not applied to such central effects. The terms "muscarinic effects" and "nicotinic effects" apply only to those symptoms resulting from *injected* acetylcholine which can be mimicked by muscarine and nicotine.

The most blatant early symptoms of carbaryl poisoning are produced by parasympathetic stimulation and include defecation, urination, lachrymation, contraction of the pupil, slowing of the heart, and drop in blood pressure. These muscarinic effects may be antagonized by atropine. Atropine competitively blocks acetylcholine, but only at muscarinic receptors. Consequently, to the extent that carbaryl poisoning involves action at muscarinic sites, atropine is an excellent antagonist. Its effectiveness varies greatly with species and in mammalian species is least effective in the mouse. Species variations may be due to the variations in the contributions of muscarinic effects to the poisoning process.

Nicotinic effects involving the neuromuscular junction can also be produced. The nicotinic effects are first progressive, excitatory, leading to twitching of the muscles, then inhibitory, leading to paralysis. Nicotinic symptoms are ineffectively treated by oximes such as 2-PAM. Convulsions are only usual in severe poisoning, and are primarily clonic, that is, with rapid repetitive movements and are central in origin. Another important central effect is inhibition of the respiratory center of the brain.

Asphyxiation occurs because of respiratory failure: Failure may be central, via the respiratory center, or peripheral, via the respiratory muscles. The cause of death in acute poisioning such as in the determinatioin of our LD_{50} in rats is asphyxiation. The relative contribution of central effects, against which atropine is effective, and peripheral effects varies with species (De Candole *et al.* 1953; O'Brien, 1960).

Cholinesterase declines proportional to dose with respiratory collapse usually not occurring until blood acetylcholinesterase is 95% inhibited (Nachmansohn and Feld, 1947). Acetylcholine in the brain rises to 2- to 3-fold in acute carbaryl poisoning.

Large quantities of atropine are required to be effective in human poisoning. Doses of 2 mg at half-hour intervals are usually administered. Efficacy is measured by the appearance of symptoms of mild atropinization in the form of dry mouth and skin. Dilated pupil is an inadequate index for measuring systemic atropine response. The 2 mg dose

commonly administered half-hourly in mild cases, or every 3-8 minutes in severe cases. Atropine is very poisonous compound and close attention to symptoms is required.

There are several competing forces at work determining the effective toxicity of carbaryl. Absorption is a very important component of carbaryl biokinetics. It is likely that many of the seemingly apparent differences in toxicity reported in the literature are due in large part to differences in absorption rate. For example, a 20 mg i.v. dose can kill a rat (Carpenter *et al.,* 1961) but 10 times that amount is required as an oral gavage dose (Bushy Run Research Center, Project Report 46-96, August 25, 1983)and 30-50 fold greater when fed in the diet.

Some destruction of the anticholinesterase activity of carbaryl probably occurs in the stomach and intestine. The half-life of carbaryl was reported as 6.4 minutes in the empty intestine (Hwang and Schanker, 1974). Carbaryl is absorbed more rapidly in starved animals than in animals allowed to eat *ad libitum.* Rodents absorb carbaryl mixed in food less rapidly than dogs because of eating habits.

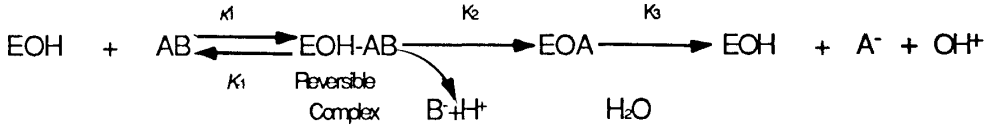
The intestine is supplied with a large amount of blood. The blood rapidly mixes with the carbaryl absorbed. The first detoxification reactions of consequence occur in the blood. The blood contains several elements which also tend to neutralize the potential anticholinesterase effects of carbaryl on the nervous system. The blood contains aliesterases, plasma and red blood cell cholinesterase. These enzymes rapidly "soak up" and metabolize carbaryl minimizing availability and effect on nervous system acetylcholine.

In the presence of very large quantities of carbaryl, first order kinetics are replaced by zero order and some free carbaryl will exists. The liver and other organs then become more important in reducing the risk to the nervous system. Carbaryl which has escaped the scavenger effects of the blood and first pass metabolism by the liver is available to inhibit the acetylcholinesterase of the nervous system.

The final line of defense is the ability of inhibited enzyme to reactivate. Reactivation is the most important factor resulting in limited carbaryl toxicity. The ability of mammals to tolerate enormous continuous exposures of carbaryl in the feed dramatizes this point. An understanding of the role that cholinesterase plays in detoxifying carbaryl is essential to understanding the toxicokinetics.

Carbaryl reacts with, and is hydrolyzed by, cholinesterase in a pattern analogous to acetylcholine. First there is the formation of the carbaryl-cholinesterase complex; second there is carbamylation of the enzyme by carbaryl; finally decarbamylation occurs freeing the enzyme and reactivating the acetylcholinesterase. The common urinary metabolite 1-naphthol is released during the second step. CO_2 is the final product of step 3.

Let us assign the symbol A for the methyl carbamoyl and B for 1-napthol and EOH will signify cholinesterase

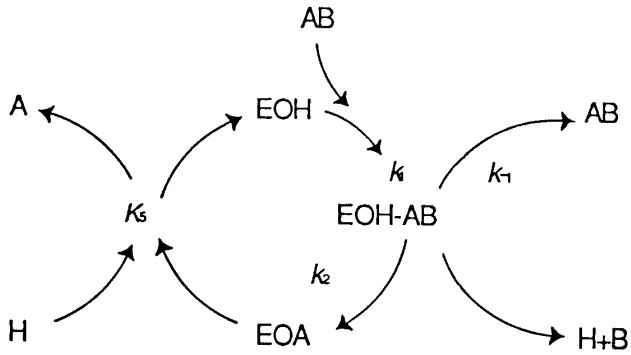


EOH-AB is formed almost instantaneously. The carbaryl-cholinesterase value for K_{-1} /K_1 , also called Ka or the affinity constant, is quite small at $1.1x10^{-5}$ per minute. Therefore, maintenance of EOH-AB is favored. K_2 is about 1.3 per minute and K_3 is slow about 0.05 per minute. These kinetics lead to the existance of small levels of the complexed carbaryl-enzyme, EOH-AB and large levels of carbamoylated enzyme, EOA. In the absence of carbaryl the enzyme recovers quickly by the reversal of EOH-AB and decarbamylation. K_3 of 0.05 per minute translates into a half-life of decarbamylation of 40 minutes.

It should be noted that, although the carbamylated form EOA is distinguished from the reversible form EOH-AB, EOA decarbamylates, by hydrolysis, to give rise to the original enzyme. Carbamylation therefore *appears* to be reversible from the point of view

of the enzyme; however, it is not reversible from the point of view of the carbaryl, which is cleaved and loses its anticholinesterase potency in the process.

The binding affinity of carbaryl and cholinesterase, as is true with other reversible inhibitors, is reduced by dilution and increasing substrate concentration. Carbaryl differs from reversible inhibitors in that the reaction is also progressive with time. The formation of EOA is clearly time dependent. EOH-AB is formed almost instantly. k_1 is very fast, whereas k_2 is relatively slow. Total inhibition of enzyme is seldom encountered. Excess acetylcholine substantially blocks fresh EOH-AB formation and the enzyme begins to recover by decarbamylation. As soon as decarbamylation occurs the EOH produced is promptly complexed.



k_1 - k_{-1} is so fast that we can neglect EOH. Steady state is approached when k_2 and k_3 steps are equal. The two rates are, k_2 [EOH-AB] and k_3[EOA] Brackets indicate concentrations. The steady state is reached when:

$$k_3[\text{EOA}] = k_2[\text{EOH-AB}]$$

Experimental values for $k_2 = 20k_3$. Steady state is reached when [EOA] = 20[EOH-AB], that is, when about 95% is in the carbamylated form and 5% in the reversible form. This is consistent with actual conditions of acute poisoning.

Comprehensive characterization of the tissue esterases involved in the hydrolysis of carbaryl remains to be completed. Albumin esterases are responsible for some of the hydrolysis of carbaryl, but they are not as active as pseudocholinesterases, aliesterases, and arylesterases. The complexity of hydrolysis has been emphasized in studies of esterases from American cockroaches, mouse, and human brain. Esterases electrophoretically separated following tissue homogenization were incubated with carbaryl as the substrate (Matsumura and Sakai, 1968; Sakai and Matsumura, 1968). A characterized aliesterase and several arylesterases of cockroach origin were also found to hydrolyze carbaryl. Mouse and human brain esterases are active toward carbaryl but may be different since the patterns of metabolites were different (Matsumura and Sakai, 1968; Sakai and Matsumura, 1968; Sakai and Matsumura, 1971).

Both the methyl and carbonyl carbon of carbarmoyl are oxidized to CO_2 (Knaak *et al.*, 1965). Krishna and Casida (1966) demonstrated that between 60 and 80% of carbaryl ^{14}C appeared as $^{14}CO_2$ when metabolized by rats. Others have reported values between 39 and 65%. Carbaryl was demonstrated to be partially metabolized to carbon dioxide *in vivo* and *in vitro* (Palut *et al.*, 1970; Hassan *et al.*, 1966). Expired radioactive CO_2 reached its peak 30 to 40 minutes after oral administration of carbonyl labeled with ^{14}C at the carbaryl group to rats (Casper and Pekas, 1971).

Several points need to be highlighted:

1. Both EOH-AB and EOA contribute to inhibition of the enzyme, but EOA will result in the destruction of carbaryl.

2. Clearance of free carbaryl from the blood by the liver will reduce carbaryl concentration and result in EOH-AB being reversed, freeing additional carbaryl to be metabolized.
3. Increasing concentrations of acetylcholine at the synapse will competitively reactivate acetylcholinesterase and release carbaryl from the EOH-AB complex.
4. Decarbamylation half-life is about 40 minutes and is a very important detoxification mechanism accounting for 60 to 80% of the metabolism relevant to the destruction of anticholinesterase activity.

Biotransformation of carbaryl by enzymes other than esterases is an important variable that also modulates the relative toxicity of carbaryl. There are a number of excellent reviews describing the biotransformation of carbaryl including those that have been prepared by Casida, 1970; Ryan, 1971; Kuhr and Dorough, 1976; Matsumura, 1975; Fukuto, 1972; Kulkarni and Hodgson, 1980. The biochemical background of the mechanisms involved is also available (Wilkinson, 1976). The biotransformation of carbaryl by enzymes other than blood esterases will not be in great detail in this monograph. The liver's role in biotransformation of carbaryl under different dosage regimes and in different species is also important.

Studies of *in vivo* pharmacokinetics are conducted in environments artificially fortified with cofactors and cations and optimized for temperature and pH. *In vitro* reactions poorly mimic the *in vivo* reality which varies with age, sex, strain, health status, diet and many other factors. The intact organism, providing the enzyme utilized in *in vitro* tests, may in fact metabolize carbaryl by an entirely different route and/or rate. The same major pathways may be qualitatively present in plants, insects, microorganisms, and vertebrates, however the rate of *in vivo* biotransformation in insects and in the plant host is usually slow compared to the rate of metabolism in non-target vertebrate species.

All species, studied and available for review, biotransform carbaryl into more polar forms enhances permitting excretion and transport away from target CNS molecules. Detoxification mechanisms have a widespread distribution among various species of plants and animals. Carbaryl is a substrate for a wide variety of enzyme-catalyzed reactions. The principal mechanisms are hydrolysis, oxidation, and conjugation.

The quantity, placement and availability factors of essential to reactions regulate the activity of tissue enzymes and contribute to the many species differences in toxicity. Carbaryl is readily hydrolyzed in the rat, sheep, dog and guinea pig *in vivo,* but less so in the monkey and pig (Krishna and Casida, 1966; Knaak and Sullivan, 1967; Knaak *et al.,* 1968). Human liver and other tissues readily hydrolyzes carbaryl (Strother, 1970; Sullivan *et al.,* 1972; Chin *et al.,* 1974). It has been reported that unmetabolized carbaryl is excreted in the urine of cows (Whitehurst *et al.,* 1963), but this has not been confirmed.

Oxidation reactions are carried out by the cytochrome P-450 family of related microsomal monooxygenase enzymes. Detailed descriptions of the various oxidation pathways for carbaryl metabolism can be found in Kuhr and Dorough, 1976; Matsumura, 1975; and Menzie, 1969. Reactions observed with carbaryl include ring hydroxylation and oxidation of the side chain. Carbaryl undergoes hydroxylation of the N-methyl group, direct ring hydroxylation and the formation of an epoxide. The oxidation proceeds thru an intermediate followed by the destruction of the intermediate either nonenzymatically or by the enzyme epoxide hydrase to form a trans diol or a hydroxide. Several oxidative routes of biotransformation are likely to function simultaneously.

In conjugation reactions, a functional group on the original or substituted carbaryl molecule is enzymatically reacted to form a water-soluble products (Ryan, 1971). Specific products formed, of course, depend upon the species but, generally can be classified as ethereal sulfates, glucuronides, glucosides, amino acid conjugates, acetylated amines, and mercapturic acids. The reactions of major importance in animals are conjugation with sulfate, glucuronide and the mercapturic acid (Knaak, 1971). Although carbaryl reactions have been widely studied (Ryan, 1971; Knaak, 1971) few actual conjugates have been

isolated from urine and feces. The compounds that have been identified have usually been cleaved by acid hydrolysis or by the enzymes sulfatase and glucuronidase.

Carbaryl is metabolized by the gut flora and tissue during absorption from the gastrointestinal tract. 1-Naphthol glucuronide is synthesized and transported to the serosal surface with mucosal ratios reported as high as 6 to 1 (Pekas and Paulson, 1970; Pekas, 1971). The half-life of carbaryl was reported as 6.4 minutes in the intestine and 2.6 minutes in the lung (Hwang and Schanker, 1974). Following intratracheal injection, ^{14}C-carbaryl, activity in the blood of rats reached its maximum in 2 to 5 minutes. Within 3 days 90% of the activity was recovered in the urine and only 2 to 5% in the feces (Nye and Dorough, 1976).

Carbaryl induced liver microsomal enzymes of chickens when administered at a dosage of 400 mg/kg/day for 5 days but produced little or no induction at half that dosage. Induction returned to near normal 11 days after dosing was discontinued (Puyear *et al.,* 1970). When carbaryl was administered for a longer period, induction occurred at dosages of 100 mg/kg/day and above but not at 50 mg/kg/day or below (Puyear and Paulson, 1972). Carbaryl is a weak inducer of hepatic microsomal enzymes in mice but 1-naphthol did not have this effect when fed at the same molar concentration in the food (Cress and Strother, 1974). Carbaryl can be toxic to the liver under some circumstances. Carbaryl, presumably of Soviet manufacture, was reported to not only inhibited cholinesterase but also to cause changes in various liver function tests and changes in the histology of the liver when administered to rats and rabbits at dosage levels as low as 0.76 and 0.38 mg/kg/day (Kagan *et al.,* 1970).

Metabolites of carbaryl are excreted in the urine and bile. An intravenous dose of 0.1 mg of ^{14}C-carbaryl resulted in recovery of 50% of the ^{14}C in the bile within the first 6 hours. At higher dosage levels smaller percentages were recovered. At 1.0 mg, which killed some rats, only 17% of the activity was recovered in the bile (Bend *et al.,* 1971). These data indicate that the i.v. lethal dose is about 200-600 times less than the oral dose. Bend *et al.* (1971) studied water-soluble metabolites in the urine and bile of rats that had received intravenous or intraperitoneal doses of ring-labeled or carbonyl-labeled carbaryl. They presented evidence for metabolites that could be hydrolyzed by acid but not by enzymes to thioethers, specifically *S*-(4-hydroxy-1-naphthyl) cysteine and *S*-(5-hydroxy-1-naphthyl) cysteine. Rats with a bile cannula were given an oral dose of carbaryl and within 48 hours they excreted 45.4% of the dose in their bile, 42.3% in their urine, and 1.4% in their feces (Marshall and Dorough, 1979).

Stimulation of the rate of biotransformation of carbaryl reduces anticholinesterase activity. Mice pretreated with the liver microsomal inducer phenobarbital were less susceptible to carbaryl poisoning. Conversely SKF 525-A, a microsomal enzyme inhibitor, made mice more susceptible (Neskovic *et al.,* 1978). As might be predicted, some carbon derived from carbaryl is bound to microsomal proteins, and the degree of binding is increased by pretreatment with phenobarbital and other inducers (Miller *et al.,* 1979).

There are several compartments to consider when modeling carbaryl toxicity.

1. Absorption - The rate of absorption from the gastrointestinal tract is rapid in starved animals and slower in animals fed *ad libitum* with a 6 minute half life for an empty stomach and 30 minute in the presence of food.
2. Esterase Compartment - The blood and liver contain a variety of enzymes which react with carbaryl. These enzymes include albumin carbamatesterase, aliesterase, cholinesterase and other esterases. Decarbamylation is an important reaction, detoxifying 60 to 80% of carbaryl. The half life of this reaction has been calculated to be 36 minutes.

3. Liver Compartment - The liver mixed function oxidases are important in detoxifying carbaryl and accounts for 20-40% at high doses and the majority at low doses. The half life is assumed to be 60 minutes.
4. Nervous System Cholinesterase - Acetylcholinesterase of the nervous system, especially the CNS is inhibited after blood and liver esterases. Respiratory collapse due to oral exposures occurs when 95% of the blood cholinesterases are inhibited. High concentrations of carbaryl are necessary to maintain inhibition of nervous system acetylcholinesterase in part because the accumulating acetylcholine acts as a reactivator.
5. Deep Compartment - A deep compartment in mammals will tolerate a 100 mg/kg oral exposure carbaryl without causing respiratory collapse. The deep compartment is primarily inhibited non CNS enzyme.

Table I-1 was constructed to provide the kinetic data needed to model several variables which might predict acute toxicity.

TABLE I-1. Percent Administered Dose Detoxified.

	Time After Administration (min)									
Half-Life (min)	6	12	18	24	30	36	42	48	54	60
6	50	75	88	94	97	99	99			
12	29	50	64	75	82	88	91	94		99
18	21	36	50	60	68	75	79	84	88	
24	16	29	40	50	57	64	70	75	79	82
30	13	24	34	42	50	56	61	66	70	75
36	11	21	29	36	44	50	55	60	64	68
60	8	13	19	23	29	34	38	42	46	50

If the dose administered x % not detoxified at any selected time - 100 mg is greater than zero respiratory collapse could occur. D - 100 mg - decarbamylated - liver metabolism > 0 = acute toxicity.

Inhibition of the acetylcholinesterase of the nervous system (not erythrocyte or plasma cholinesterases) is the biochemical lesion by which carbaryl neurotoxicity is produced. Several theories have been proposed over the years concerning possible secondary mechanism(s) by which carbaryl exerts toxicity. Most investigators agree that acetylcholinesterase of the CNS is the target and significant inhibition first results in overt neurotoxicity which is then followed by dysfunction of other controlled systems and organs.

Because of its central role the manner by which carbaryl inhibits cholinesterase will be recapitulated. There are two distinct steps in the reaction of carbaryl with the acetylcholinesterase molecule. Acetylcholinesterase first couples with carbaryl to form an intermediate which can reversibly dissociate, releasing free enzyme and carbaryl or be decarbamylated. The affinity constant is low and dissocciation proceeds rapidly. The complex decomposes into a stable carbamylated enzyme with the loss of the 1-naphthol. The carbamylated enzyme then hydrolyzes the mono-methyl carbamic acid which is unstable and degrades to carbon dioxide and methylamine (Krishna and Casida, 1966; Aldridge, 1971). Decarbamylation of carbaryl carbamylated esterases is reasonably rapid. Carbaryl is a relatively poor substrate for acetylcholinesterase and yields an appropriately low turnover rate. In the absence of a large excess of carbaryl, acetylcholinesterase will

recover quickly by reverse dissociation of the complex and decarbamylation. The half-life for the decarbamylation has been variously calculated to be 30 to 40 min (O'Brien, 1967; Reiner and Aldridge, 1967). Typically, without the presence of an excess of carbaryl, the enzyme will begin to recover within a few minutes and would be completely reactivated after a few hours. Experiments have demonstrated that carbaryl is not tighly bound to the active site and can be dissociated easily by dilution or by increasing the acetylcholine concentration.

The absence of a tenacious carbaryl bond to the enzyme fortunately reduces toxicity but creates a number of problems in the analysis of inhibited cholinesterase and the enzymes quantitative measurement as an index of exposures. Reports in which obvious cholinergic signs of toxicity are observed but the reported analysis indicate little inhibition of cholinesterase are obviously flawed. As previously discussed, difficulty may arise from the improper selection of the enzyme to be assayed, with plasma pseudocholinesterase being less sensitive to carbaryl than the erythrocyte acetylcholinesterase. In addition, the analyst must have considered ease with which the carbamylated cholinesterase spontaneously reactivates following dilution, lysis (in the case of erythrocytes) or the addition of substrate. Finally, the rate at which carbaryl has been degraded *in vivo* must be considered requiring rapid sampling and appropriate stabilization in storage. Analytical methods must have adequately addressed all of these problems before the results reported are useful or comparable.

Treatment

The symptoms associated with carbaryl toxicity are due to the accumulation of the neurotransmitter acetylcholine at the nerve endings of the parasympathetic and sympathetic autonomic ganglia, the postganglionic parasympathetic nerve endings, and at the neuromuscular junctions of the somatic motor nerves. Atropine is the antidote of choice for carbaryl poisoning. Atropine antagonizes the action of acetylcholine by blocking the receptor sites. The predominant action of atropine is directed toward the postganglionic, parasympathetic nerve fibers innervating exocrine glands, gastrointestinal tract, respiratory tract, eyes, bladder, and heart. Atropine also exerts a remarkable central effect, appearing to have some direct action on the respiratory center.

Ideal treatment with atropine involves administering frequent and small doses (0.5 to 2.0 mg) intravenously until there is a dilation of the pupils and the face becomes flushed and/or sweating disappears (Namba *et al.,* 1971). The physiological signs of the patient should be carefully monitored and atropine administration carefully titrated. Excess atropine can cause severe toxicity, especially in a compromised patient.

2-PAM reactivates the nicotinic site on acetylcholinesterase. Carbaryl acts primarily on muscuratic sites. The use of specific oxime antidotes such as 2-PAM (2-methylpyridine-2-aldoxime chloride or pralidoxime chloride) is contraindicated in carbaryl poisoning. The scientific basis for this position is found in three publications. Two studies involving carbaryl toxicity in dogs and rats, report the protective effect of atropine was reduced by the concomitant administration of 2-PAM (Carpenter *et al.,* 1961; Sanderson, 1961). The acute toxicities of eight anticholinesterase carbamates, including carbaryl were examined in the male rat. Atropine reduced the observed toxicities of all of the agents. The oximes, obidoxime (Toxogonin, oxybis - [4 - hydroxyiminomethyl-pyridinium - 1 - methyl] dichloride) and P2S (2 - hydroxyiminomethyl - N - methylpyridium methyl methanesulfonate), in combination with atropine, enhanced the therapeutic efficacy of atropine with the exception of carbaryl where oximes enhanced toxicity. Obidoxime markedly reduced the protection afforded by atropine. In a report involving a suicide attempt by drinking carbaryl, it was noted that the patient's condition deteriorated rapidly after the administration of 2-PAM (Farago, 1969). (See VI: Human Exposure). The 2-PAM contraindication should not be extended to include all other carbamate insecticides.

Central Neurotoxicity

Azizova (1976) administered carbaryl of unknown purity to rabbits at a dose reported to be 1/100, 1/50 and 1/10 of the LD_{50} (LD_{50} is approximately 800 mg/kg in these workers' laboratory) for six months. Azizova reported CNS changes observable 45 days after cessation of dosing at all levels, with lower doses resulting in glial fibrosis and dystrophic changes in the villi epithelium. Higher doses caused changes in vessel walls interpreted as vasculitis as well as diffuse damage to the basal ganglia. Azizova interpreted these findings as indicating that continued exposure to carbaryl resulted in reversible changes at lower doses and persistent changes similar to meningoencephalitis at higher doses.

A 90-day toxicity study (Dikshith *et al.* 1976) in male rats orally dosed with 200 mg of carbaryl/kg 3 days/wk is worthy of mention. Surprisingly, this extreme dose was reported as not producing any overt toxicological signs. No histological changes were noticed, but as expected brain cholinesterase activity was significantly inhibited.

Boyd and Boulanger (1968) reported that congestion of the brain and meninges was the most common gross pathologic finding in rats acutely killed with carbaryl.

Behavioral Effects

Many reports in the toxicological literature document acute effects after administration of cholinergic drugs, such as carbaryl, on animal learning and performance of well defined tasks. Drugs that potentiate the action of the neurotransmitter acetylcholine (*e.g.,* physostigmine), if administered shortly after learning, generally facilitate learning (Squire, 1976) and support a theory of learning which holds that remembering and forgetting are results of time-dependent changes in cholinergic synapses (Deutsch, 1971). Cholinergic drugs often appear to have little effect on performance in experiments designed to emphasize well learned behaviors over acquisition of new behaviors. This is especially true for tasks using a positive reward (*e.g.,* food), unless a sufficient dose level is administered to suppress all behavioral responses (Vaillant, 1964). In well-learned tasks involving avoidance of negative rewards (*e.g.,* footshock), cholinesterase inhibitors in large doses eliminate responding, while in lower doses they reduce but seldom abolish the avoidance behavior (Funderburk and Case, 1947). The literature reporting behavioral experiments utilizing carbaryl is in general agreement with the results obtained for similar tasks using more widely studied cholinesterase inhibitors. Therefore there is nothing to suggest carbaryl evokes unique behavioral effects.

Goldberg *et al.* (1965) determined that a single dose of 8.0 mg/kg i.p. carbaryl to the rat reduced avoidance response by 50% for 30 mintues, while a dose of 7.3 mg/kg i.p. was required for a 50% reduction of a positive reward response (*e.g.*, food).

Yakim (1967) reported that cats inhaling carbaryl at a concentration of 40 mg/m^3 showed decreased responsiveness to a classical food reward conditioning paradigm, but this deficit was pronounced only immediately following the first administration of carbaryl. It is reasonable to speculate that the early transient effect was a display of simple cholinergic mediated distress.

Singh (1973) found that spontaneous locomotor activity in the running wheel was reduced during the 60 minutes following acute administration of 0.56 or 2.24 mg/kg carbaryl. Similar effects were found for rats receiving atropine sulfate, while concurrent administration of atropine and carbaryl resulted in no greater decrease in wheel running than administration of carbaryl or atropine alone. In the same study, daily administration of 2.24 mg/kg carbaryl i.p. for 14 days was without effect on the number of wheel revolutions recorded daily. These data are interpreted by the author as indicating cholinergic mediation of wheel running behavior. The data might also be interpreted to indicate that both drugs produce brief generalized nonadditive distress resulting in decreased spontaneous activity.

Desi *et al.* (1974) studied the neurotoxic effects of carbaryl in subchronic experiments with rats. Doses were 1/40th and 1/80th of the oral LD_{50} (the LD_{50} is estimated as 800 mg/kg) in the diet feeding for 50 days. Disturbances were found in various functions of the central nervous system. Rats had increased difficulty in performing tasks and forgot previously learned skills. Irritability increased proportional to the dose administered. The authors expressed the opinion that carbaryl may have had an effect on brain structures responsible for both high and low excitatory levels, perhaps through stimulating and inhibiting the neurons. Brain acetylcholinesterase activity was inhibited to varying degrees depending on the region. The protein content of various portions of the brain, except the cerebral cortex, increased in significant amounts. Organ weights were not significantly altered except for the adrenals. This was though due to a nonspecific stress effect, as shown by Hassan (1971) and Hassan and Cueto (1970). No histologic lesions were described.

Administration of phenobarbital or chlordiazepoxide with carbaryl yielded effects on both positive and negative reward which were additive and suggest an independence of their respective mechnaisms of action. The antidepressant effects of imipramine attributed to central anticholinergic activity. Co-administration of imipramine diminished the effect of carbaryl administration, while chlorpromazine administration yielded more than additive effects in the absence of additional inhibition of brain acetylcholinesterase. The antidepressant effects of imipramine have been attributed to central anticholinergic activity. The augmenting effects of chlorpromazine may be due to a central adrenergic blocking effect of the tranquilizer inhibiting the ergotropic and exacerbating the cholinergic effects of carbaryl.

Albright (1977) studied the acute intoxication of rats with 10 mg/kg carbaryl and found an increase in spontaneous locomotor activity in a familiar environment, while reducing exploration in a novel environment.

Viter (1978) exposed rats to a concentration of 12 - 23 mg/m^3 of carbaryl for 4 hours daily for 4 months. The rats were found to perform a maze task for a food reward more slowly during the exposure period than an untreated control. A schedule of exposures on alternate weeks during a four month period likewise resulted in slower performance during the last month of exposure when treated were compared to controls. When the interval between exposure and no exposure was increased to 2 weeks and alternated for four months, treatment differences disappeared. These data suggest that maze deficits are a product of acute intoxication with carbaryl rather than evidence of a persistent behavioral change resulting from poisoning.

Memory impairment has been described as a general effect due to exposure to carbaryl however few studies have investigated the effects of carbaryl specifically on memory in animals. Heise and Hudson (1985a) reported that carbaryl did not selectively disrupt working memory as measured in three continuous delayed response procedures. In general, as the i.p. doses of carbaryl increased (from 2.5 to 5.0 mg/kg), only small decrements in accuracy were observed. A higher dose (10 mg/kg) produced an abrupt decline. In contrast, the prototypic amnesic agent, scopolamine, did selectively disrupt working memory under the same test conditions.

The general applicability of the study by Heise and Hudson (1985a) was limited by its exclusive use of delayed response procedures. In delayed response procedures the stimulus events presented prior to the delay (retention) interval completely determine which post-delay response will be correct. It is theoretically possible for the animal to "bridge the gap" by means of orienting responses (Fletcher, 1965). Furthermore, only a single (5 sec) intertrial (retention) interval was employed. Consequently any specific effects of treatments on retention could not have been detected.

Heise and Hudson (1985b) compared carbaryl and scopolamine and physostigmine, as in the previous study, but measured their effects on working memory in a delayed comparison (sometimes called delayed discrimination) procedure and continuous non-match. In a delayed comparison procedure the correct post-delay response is

determined by post-delay as well as by pre-delay stimuli (Heise and Hudson, 1985a); hence the likelihood of coding by means of orienting responses is less than in delayed response. In addition, the continuous non-match schedule included three different intertrial (retention) intervals of 2.5, 5, and 10 seconds, thus making possible the assessment of treatment effects on the time-dependent process of retention.

Carbaryl and physostigmine had similar effects on continuous non-match performance. The percentage of hits and false alarms paralleled dose, falling from control levels to near total response failure within two doublings of the dose. The magnitude of effects did not vary consistently with intertrial (retention) interval. Effects of these same compounds on performance of the Small-Difference discrimination (for which the baseline accuracy was equivalent) were qualitatively and quantitatively similar to their effects on continuous non-match performance. Carbaryl and physostigmine also disrupted performance of a Large-Difference discrimination (for which the trial stimuli were the same) to approximately the same extent that they disrupted performance on the continuous non-match and on the Small-Difference discrimination. Control performance is substantially more accurate in the Large-Difference discrimination than in the Small-Difference discrimination. Carbaryl and physostigmine had similar effects on performance of both discriminations.

It is concluded that carbaryl and physostigmine did not selectively impair working memory but indiscriminately suppressed all behavior. If working memory had been specifically affected, there should have been a range of doses at which the animals continued to perform (although inaccurately) in the continuous non-match, and carbaryl and physostigmine should have affected memory for the trial stimuli more than it affected discrimination. Neither of these effects were obtained. Scopolamine, an alleged amnesic agent, has been shown to produce precisely these effects on continuous non-match performance in experiments carried out under similar conditions by Pontecorvo(1983), Spencer (1985), and Heise and Hudson, (1985).

Carbaryl produces behavioral toxicity only at doses producing overt neurotoxicity.

Effects on EEG

Several investigators have anticipated effects due to prolonged low dose carbaryl administration. Gross brain function as detected by EEG was suggested because of the apparent cholinergic stimulation seen following acute administration of high doses of carbaryl.

Belonozhko and Kuchak (1969) obtained EEG recordings from cats fed 35 mg/kg/day carbaryl for 90 days and were unable to identify any change in cortical rhythm for the parameters measured.

Wills *et al.* (1968) studied the effects of daily p.o. doses of carbaryl (0.06 or 0.12 mg/kg) in male human volunteers. After 6 weeks of exposure, no EEG changes were found which were attributable to carbaryl exposure.

Santolucito and Morrison (1971) fed rhesus monkeys a "low-level" of carbaryl for 18 months. They periodically obtained EEG recordings from three surface electrodes. Analysis was by visual examination of the tracings. No differences in the basic pattern of EEG were seen between treated and control animals. There was no dose response relationship. The authors concluded that a quantitative, but not a qualitative, difference in EEG was observed and that the mangitude of differences was so small it did not permit elaboration.

Desi *et al.* (1974) administered 1/40th and 1/80th of an oral LD_{50} (LD_{50} is approximately 800 mg/kg) in the diet for 50 days and reported EEG deviation proportional to the dose administered.

Changes in EEG have only been detected at overtly neurotoxic doses.

Peripheral Neurotoxicity

A number of compounds possessing anticholinesterase activity have been implicated as causative agents in producing neuropathology of the peripheral nervous system of the type classically associated with tri-*ortho*-cresyl-phosphate (TOCP). Early investigators characterized these delayed peripheral neuropathies as peripheral demyelination, labeling the phenomena for the readily apparent changes in the myelin sheath in the absence of a knowledge of the initiating phenomena. Inability to identify the underlying etiology led to the early policy of routine screening of anti-cholinesterase compounds for TOCP-like "delayed neurotoxicity" (Cranmer and Hixson, 1983).

The classic protocol for such screening utilizes administration of large doses to the chicken. Histological examination of selected peripheral and central nerves follows after sufficient time has passed delay to permit appearance of this slowly developing phenomena. This general protocol was employed by Carpenter *et al.* (1961) to survey the paralytic potential of carbaryl. No consistent histological changes were observed even though doses from 250 to 3,000 mg/kg were utilized. Doses above 1,000 mg/kg resulted in transitory leg weakness, which the authors attributed to the direct cholinergic effects of the compound. A similar protocol executed by Gaines (1969) yielded comparable results.

In contrast to the studies in chickens (Carpenter *et al.* 1961), neuromuscular changes were observed during a chronic dietary study in swine fed 150 and 300 mg of carbaryl/kg/day body weight for 8 to 12 wks (Smalley *et al.* 1969). The development of toxic signs at these very high dose levels was not surprising and progressed following a consistent pattern. Exposed animals were not anorexic, but signs of incoordination, ataxia, weakness of suspensory ligaments, staggering gait, and prostration developed, resulting in death within 2 to 3 days after symptoms began. When animals were placed on normal feed, clinical symptoms regressed in 9 to 11 days. One characteristic sign observed in treated pigs was discoloration of voided urine following exposure to air and light which stained the concrete floors a brown-black color. Pathologic lesions were noted in skeletal muscle and in the myelinated tracts of the brain stem and cerebellar peduncles of the central nervous system. Three distinct forms of myopathy were identified. These were discrete myodegeneration of a traumatic or ischemic type, acute hyaline and vascular myodegeneration with discontinuous regenerations, and acute degeneration associated with dystrophic calcification. A primary vascular disturbance was related to changes in the central nervous system. Vascular degeneration was identified by endothelial hypertrophy, hyalinization of the walls, hemorrhage, fluid accumulation in the white matter, edema, and astrocytic gliosis. In summary, no consistent histological changes in peripheral nerves were evident, although skeletal myo-degeneration and vasogenic edema were observed.

Santolucito and Whitcomb (1971) utilized the isolated soleus muscle of the rat *in situ* to measure the acute mechanical response of the muscle to oral administration of 56 mg/kg of carbaryl. Two hours after dosing, the tension developed during complete teta - nus was found to be increased and the time constant of tension development decreased.

Miller *et al.*, (1973) using a repeated dose of 125 mg/kg, was unable to produce paralysis in dogs, but did report paraplegia in swine fed the same dose level.

Carbaryl does not produce delayed neuropathology in experimental animals similar to the organophosphate reduced neuropathics observed in humans and the most relevant experimental animal models.

Neurochemistry

Intraperitoneal dosages of carbaryl as low as 5 mg/kg can produce a hyperglycemic response in intact or hyphophysectomized but not in adrenalectomized rats. Although 5

mg/kg produced little or no clinical cholinergic effects, it does produce a measurable decrease in brain cholinesterase (Orzel and Weiss, 1966).

Repeated oral dosages in excess of 1 mg/kg/day lead to increased levels of serotonin in the blood and brain of rats in 2 to 4 months followed by a decrease after 6 months of dosing (Butygin and Viatchannikov, 1969).

Soviet researchers have placed special emphasis on the effects of carbaryl on cellular permeability, glycolysis, the state of proteins, tissue respiration, and oxidative processes. These matters have been reviewed by Khaikina and Kuz'minskaia (1970). In spite of extensive study, it remains unclear whether the changes reported are causes or effects and especially whether they are associated with carbaryl or with some impurity.

3-Methoxy-4-hydroxymandelic acid is a normal constituent of urine, but rats excrete about three times the usual concentration when fed carbaryl at a dietary concentration of 700 ppm for a month. The concentration approached normal after 195 days of feeding, and a dietary level of 100 ppm was without effect. The higher dosage caused a 68% increase in the turnover rate of norepinephrine in the heart but no change in the steady state concentration of the transmitter (Hassan, 1970, 1971). Other studies have confirmed that sufficient dosages of carbaryl constitute a stress situation (Dyadicheva, 1971). However, excretion of norepinephrine, total metanephrines, and 3-methoxy-4-hydroxymandelic acid were not increased in workers exposed to carbaryl and other insecticides, and their excretion of epinephrine was significantly lower compared to controls (Richardson *et al.*, 1975). The contradiction may be due to species differences but is more likely a response to dosage.

Changes in catecholamine have been associated with sub-lethal single doses and repeated administration of carbaryl. Hassan and Santolucito (1971) demonstrated an increase in brain levels of serotonin and its primary metabolite, 5-hydroxy-3-indoleacetic acid, when rats were given 60 mg/kg as a single oral dose. The authors suggested that the result might be secondary to stress.

Ahdaya *et al.* (1976) demonstrated a decrease in body temperature of mice exposed to carbaryl and suggested that the regulatory mechanisms responsible for maintaining body temperature were affected.

Burisian and Edens (1978) studied neurochemical parameters in Japanese quail given single i.m. injections of 30 mg of carbaryl/kg which affected biochemical parameters in the brain and heart. Brain norepinephrine turnover was not affected, but dopamine concentrations were elevated as indicated by an increase in dopamine synthesis. Increased norepinephrine synthesis was suggested as the cause of elevated levels of norepinephrine in the heart 48 hours post-dosing. Plasma glucose or cholesterol levels were not elevated following carbaryl administration; however, an increase in adrenal catecholamine turnover was found.

The effects of prolonged exposure to carbaryl upon neurochemical and blood chemical parameters was studied by Bursian and Edens (1979). Carbaryl was administered to Japanese quail from day of hatching to 14 wk of age at 50, 150, 300, 600, 900, and 1,200 ppm dietary levels. Treatment resulted in no changes of whole-brain acetylcholinesterase activity, plasma glucose levels, brain, heart, or adrenal norepinephrine. Brain dopamine levels were significantly elevated at the 600 and 1,200 ppm levels (no measurement was made of the 900-ppm treatment group). It was concluded that Japanese quail are less affected by carbaryl than are mammals.

Neurochemical effects are due to the direct toxic effect of carbaryl and not secondary mechanisms for the expression of toxic action.

Acute Toxicity

At least forty reports on the acute toxicity of carbaryl and formulated products exist in the literature. Early studies were often performed on non-starved animals and yield

higher LD_{50}s than contemporary reports. Toxicity progresses from i.v., i.p., oral and dermal. Females are occasionally slightly more susceptible than males. Dermal exposures are without effect. Mammalian species yield oral LD_{50}s greater than 100 mg/kg. The dose response curve is steep. Toxicity parallels carbaryl concentration. Avian species appear to be more resistant to the toxic effects of carbaryl than mammals but this is likely due to differences in absorption not nervous system resistance or increased biotransformation.

The acute oral mammalian toxicity of carbaryl is low when compared to most alternative insecticides and best appreciated by comparing the ratio of application rate to mammalian LD_{50}. Literature values for the acute oral toxicity of carbaryl in rodents vary widely with laboratory, strain, sex and species. Indeed the inherent variability of the LD_{50} as a measure of acute toxicity is obvious when comparing repeat tests under similar conditions. Values for the LD_{50} in rats have ranged from 150 mg/kg to 850 mg/kg.

Dermal LD_{50} values document the consistent lack of mortality at 2,000 mg/kg with some studies reporting doses exceeding 5,000 mg/kg with no mortality.

The toxic effects of carbaryl on skin and eyes appear to be minimal. Eye and skin irritation tests report only transient effects and no data were discovered which would justify classification of carbaryl as an eye or skin irritant. An early report suggested that administration of a petroleum oil-based carbaryl formulation SEVIN®4 Oil exhibited a slight sensitizing potential, however, other work failed to confirm the sensitizing effect.

Oral - Rats. A report on various formulations prior to the 1979 EPA guidelines stated LD_{50} values for 99% technical carbaryl, 97.5 MC, 80 DB, 95 technical, 50-W and 85-S to be 233 mg/kg, 187 mg/kg, 214 mg/kg, 259 mg/kg, 354 mg/kg and 283 mg/kg, respectively. (Carnegie-Mellon Report, 37-53, June 6, 1974.) SEVIN® liquid (44% carbaryl) produced an LD_{50} of 0.536 ml/kg (range 0.362 ml/kg - 0.793 ml/kg in non-fasted Wistar rats (Carnegie - Mellon University, Report May 16, 1975).

Data on acute toxicity of carbaryl to rats is available from the USSR literature. Female rats have been reported to be1.7 times more sensitive to carbaryl than male rats. Servituna (1964) reported an LD_{50} of 850 mg/kg and Yakim (1967) 721 (653-789) mg/kg. These values are in general agreement with the contemporary U.S. literature.

Oral - Mice. Ahdaya *et al.*, 1976 reported an LD_{50} of 588 mg/kg for albino mice. Yakim, (1967) reported an LD_{50} of 363 mg/kg. Toxicity data for mice appear in at least four references in the USSR literature cited in the United Nations Environmental Program Carbaryl Review published in 1982. The toxicity of carbaryl obtained from several sources has also been reported in the 1982 UNEP Review. The strain and origin of the white mice used was not reported. Some authors reported that they did not consider age and sex to be significant factors affecting the LD_{50}. LD_{50} values range from 175 to 600 mg/kg.

Bukin studied the LD_0, LD_{50} and LD_{100} data for a variety of carbaryl containing samples and noted the possible effects of wettable powder in increasing absorption. It is noteworthy that the slope of the lethality curve is steep with a tripling of dose progressing from an LD_0 to an LD_{100}.

Oral - Other Species. Carpenter *et al.* (1961) studied the oral LD_{50} of carbaryl in the guinea pig, rabbit, dog and cat. The LD_{50}s reported were: guinea pig - 280 mg/kg; rabbit - 710 mg/kg; dog - 250-795 mg/kg; cat - 125-250 mg/kg. Yakim (1967) reported an LD_{50} of 150 mg/kg for the cat. Smalley (1969) reported an LD_{50} for swine of 1,500 - 2,000 mg/kg. Tucker and Crabtree (1970) reported an LD_{50} for Mule deer of 200-400 mg/kg.

Carbaryl is relatively non-toxic to mammals when compared to other insecticides. There is a reasonable cluster oral LD_{50}'s from several mammlian species around 200 mg/kg.

TABLE I-2. Rat Acute Oral Toxicity of US Carbaryl and Carbaryl-Containing Mixtures Since 1979.

Test Material	Strain	LD_{50} Mean	LD_{50} Range
Technical	SD	685 mg/kg[a]	(612-767)
Technical	SD	255 mg/kg[a]	(202-321)
Technical	Wistar	264 mg/kg[b]	(168-477)
80%	Wistar	203 mg/kg)[c]	-
80%	Wistar	406 mg/kg[c]	-
43%	Wistar	0.325 ml/kg[d]	-
43%	Wistar	584 ml/kg[d]	-
43%	Wistar	0.595 ml/kg	-
40%	Wistar	0.313 ml/kg[f]	-
40∞	Wistar	0.339 ml/k[gf]	-
FR (40%)	SD	647 mg/kg[g]	(457-917)
RP-2(23%)	SD	1.82 ml/kg[h]	(1.53-2.17)
20% Bait	SD	3.25 ml/kg[i]	-

[a] Technical grade carbaryl (made in India) yielded an LD50 of 685 mg/kg (range 612 mg/kg - 767 mg/kg) when administered in methyl cellulose (CDC Research Inc. Report CDC-UC-047-79, January 1980); technical grade carbaryl (made in West Virginia) produced in LD_{50} of 255 mg/kg (range, 202 mg/kg - 321 mg/kg) (CDC Research, Inc. Report CDC-UC-046-79, January 1980).

[b] In fasted rats, technical carbaryl (99%), yielded on LD_{50} value of 283 mg/kg in males (range 168 mg/kg - 477 mg/kg), 246 mg/kg in females (range 182 mg/kg - 333 mg/kg) and in combined males and females, 264 mg/kg (Bushy Run Research Center, Project Report 46-71, July 12, 1983).

[c] SEVIN® 80 - sprayable yielded an LD_{50} of 203 mg/kg in fasted female rats and 406 mg/kg in male fasted rats (Bushy Run Research Center, Project Report 46-97, September 20, 1983).

[d] With SEVIN® XLR (43%), LD_{50} values were reported to be 0.584 ml/kg in fasted male rats and 0.325 ml/kg in fasted female rats (Bushy Run Research Center, Project Report 46-96, August 25, 1983).

[e] SEVIN® XLR-UCSF-1 (43%) yielded an LD_{50} of 0.595 ml/kg (range 0.389 ml/kg - 0.909 ml/kg). (Carnegie-Mellon Report 41-133, January 23, 1979.)

[f] SEVIN® FR (40%) produced an LD_{50} of 0.339 ml/kg.in fasted male rats and 0.313 ml/kg in fasted female rats (Bushy Run Research Center, Project Report 45-120, July 1, 1982).

[g] LD_{50} values of SEVIN® FR (40%) were reported to be 750 mg/kg, 527 mg/kg and 647 mg/kg for fasted males, females and combined males and females, respectively; 95% confidence limits were 467 mg/kg - 1202 mg/kg (males), 294 mg/kg - 947 mg/kg (females) and 457 mg - 917 mg/kg (combined); no gross histopathology was observed at necropsy (Hazelton Laboratories America, Inc., Final Report, February 10, 1982).

[h] SEVIN® RP-2 (23%) was reported to have an LD_{50}, of 1.82 ml/kg (range 1.53 ml/kg - 2.17 ml/kg). (CDC Research, Inc. Report CDC-UC-122-80, October 29, 1980.)

[i] Using a 20% carbaryl bait, the LD_{50} was calculated to be 3.25 ml/kg. The test article was suspended in methyl cellulose. Body tremors, muscle fibrillation, lacrimation, dyspnea and convulsions were noted (CDC Research, Report CDC-UC-123-80, 1980).

Limited data are available on the effects of carbaryl in avian species but reported LD_{50} values demonstrate the greater resistance of birds to the chemicals toxic effects when compared to mammals. Tucker and Crabtree (1970) reported oral LD_{50}'s for pigeons (1,000-3,000 mg/kg), Japanese Quail (2,290>5,000 mg/kg), young mallards (2,179-5,000 mg/kg), young pheasants (2,000>5,000 mg/kg), Canadian geese (1,790 mg/kg) and sharp tailed grouse (780-1,700 mg/kg). Avian speces do not readily absorb carbaryl and higher LD_{50}s should not be interpreted as unique resistance to anticholinesterase effects.

TABLE I-3. Acute Oral Toxicity of Carbaryl in Male (M) and Female (F) Mice[a]

Sample	LD_0		LD_{50}		LD_{100}		N[c]
	F	M	F	M	F	M	
Carbaryl	137.5	137.5	275.0	275.0	400.0	400.0	290
Tech. 1 Carbaryl[b]	-	150.0	-	322.5	-	462.5	70
Tech. 2 Carbaryl[b]	-	150.0	-	375.0	-	500.0	80
85% W.P. (USA)	50.0	58.9	206.6	246.0	333.3	411.8	220
50% W.P. (USA)	50.0	100.0	210.0	480.0	450.0	900.0	90
50% WP. (France)	50.0	100.0	240.0	480.0	400.0	800.0	80
50% W.P. (Germany)	50.0	100.0	175.0	350.0	300.0	600.0	60

[a]Ref: Bukin, 1966
[b]Different Batches
[c]Number of Mice

Eye and Skin. Numerous studies have demonstrated the acute dermal toxicity of carbaryl to be greater than 2,000 mg/kg (CDC Research, Report CDC-UC-008-81, 1981), (Carnegie - Mellon, Report 41-163, January 23, 1979), (Bushy Run Research Center, Project Report 46-96, August 25, 1983), (Hazelton Laboratories America, Inc., February 10, 1982). Additional studies have found no mortality at dose levels of 5,000 mg/kg or more (Carnegie - Mellon, University Report, May 16, 1975) (Carnegie - Mellon, Report 37-53, June 6, 1974).

Carbaryl and formulations thereof appear to produce no irritant effects when administered to the skin of albino rabbits. In a study that followed CPSC & EPA guidelines, SEVIN® RP-2 (23%) produced a Primary Irritation Index score of 0.44; this score places a test article in a non-irritant category (CDC Research, Report CDC-UC-009-81, 1981). No skin irritation was reported in other studies (Bushy Run Research Center, Project Report 46-71, July 12, 1983) (Bushy Run Research Center, Project Report 46-97, September 20, 1983) (Hazelton Laboratories America, Inc., February 1, 1982). Transient erythema was reported in one study, but no irritation was observed after one day (Bushy Run Research Center, Project Report 46-96, August 25, 1983).

Effects of carbaryl on the eyes, when administered to rabbits, appear to be inconsequential. SEVIN® RP-2 (23%) was reported to be a non-irritant following application [washed & unwashed eyes (CDC Research Report CDC-UC-008-81, 1981)]. Technical SEVIN® produced conjunctival irritation initially in 6 of 6 rabbits, but all were normal at 2 days (Bushy Run Research Center, Project Report 46-71, July 12, 1983). No eye irritation was reported for SEVIN® 80 sprayable (500 mg) (Bushy Run Research Center, Project Report 46-97, September 20, 1983). SEVIN® XLR (43%) produced transient iritis & conjunctival irritation but eyes were normal at 3 days (Bushy Run Research Center, project Report 46-96, August 25, 1983). SEVIN® FR (40%) produced no eye irritation in another study conducted according to EPA guidelines.

Inhalation. Lacrimation and tremors were reported following 4 hrs exposure of rats to 1,300 mg/m^3 of SEVIN® liquid (44%) (Carnegie - Mellon University, Report May 16, 1975). An earlier report on LD_{50} values of carbaryl and formulation thereof provided ranges of 5.0 mg/m^3 to 23 mg/m^3 (Carnegie - Mellon, Report 37-53, June 6, 1974). Onset of death was from 30 minutes to 1 hour following initiation of exposure (exposure time, 0 to 4 hrs). Typical signs of toxicity were noted (tremors, salivation & lacrimation). SEVIN® RP-2 (20%) produced no deaths in rats exposed to a concentration of 210 mg/m^3 although hypersalivation, dyspnea, tremors, nasal & ocular discharge were noted.

Sensitization. SEVIN® FR (40%) was found to exhibit no sensitizing potential in a study conducted according to FIFRA guidelines (Bushy Run Research Center, Project

Report 45-146, August 30, 1982). When guinea pigs were treated with technical SEVIN®, followed by a challenge dose of SEVIN® 4 Oil, a slight sensitization response was observed (Mellon Institute, Special Report 20-89, June, 1957).

TABLE I-4. Summary of LD_{50} Values by Various Routes of Exposure in Selected Species.

Species	Route	LD_{50} (mg/kg)	References
Rat, m	oral	850	Gaines, 1960
Rat, f	oral	500	Gaines, 1960
Rat	oral	510	Carpenter *et al.*, 1961
Rat	oral	850	Servituna, 1964
Rat	oral	515	Rybakova, 1966
Rat	oral	600	Coulston, 1966
Rat	oral	721	Yakim, 1967
Rat	oral	233	Carnegie-Mellon, 1974
Rat, m	dermal	>4,000	Gaines, 1960
Rat, f	dermal	>4,000	Gaines,1960
Rat	dermal	>5,000	Carnegie-Mellon, 1974
Rat	dermal	>2,000	Carnegie-Mellon, 1979
Rat	dermal	>2,000	CDC-UC-008, 1981
Rat	dermal	>2,000	Hazelton Labs, 1982
Rat	dermal	>2,000	Bushy Run 46-96, 1985
Rat	ip	200	Wilhelm and Vandekar, 1966
Rat	iv	24	Carpenter *et al.*, 1961
Rat	iv	42	Wilhelm and Vandekar, 1966
Rat	Respiratory	5 to 23 mg/m^3	Carnegie Mellon, 37-53, 1974
Mouse	oral	437.5	Rybakova, 1966
Mouse	oral	650	Coulston, 1966
Mouse	oral	275	Bukin, 1966
Mouse	oral	363	Yakim, 1967
Mouse, m	oral	108	Haley *et al.*, 1974
Mouse, f	oral	116	Haley *et al.*, 1974
Mouse	oral	588	Ahdaya *et al.*, 1976
Mouse	ip	25	Baron *et al.*, 1964
Mouse	ip	29	Balba and Casida, 1968
Guinea pig	oral	280	Carpenter, 1971
Rabbit	oral	710	Carpenter, 1971
Rabbit	ip	223	Carpenter *et al.*, 1961
Dog	oral	<500	Coulston, 1966
Dog	oral	250-795	Carpenter, 1971
Cat	oral	150	Yakim, 1967
Cat	oral	125-250	Carpenter, 1971
Swine	oral	1,500-2,000	Smalley, 1969
Deer	oral	200-400	Tucker and Crabtree, 1970
Monkey	oral	>1,000	Coulston, 1966

Conclusions

The mode of action of carbaryl is primary neurotoxicity. Carbaryl produces behavioral toxicity only at doses producing overt neurotoxicity. Changes in EEG are only detected at overt neurotoxic doses. Carbaryl does not produce delayed neurotoxicity or myelin degeneration. Neurochemical effects are due to the direct toxic effect of carbaryl and not secondary mechanisms for the expression of toxic action.

Mammalian oral LD_{50}'s cluster around 200 mg/kg. The dermal route of exposure has resulted in no mortality at 2,000 mg/kg and while mortality occasionally observed at doses exceeding 5,000 mg/kg. No data identifying carbaryl as an irritant was identified. Table I-4 provides a summary of some additional older data of the acute mammalian toxicity of carbaryl.

II: DEVELOPMENTAL TOXICITY

Summary

The developmental and reproductive toxicity of carbaryl has been studied in at least 10 different mammalian species using a wide variety of study designs and routes of administration. Developmental toxicity is defined as any adverse effect on reproductive performance, including decreased fertility, increased prenatal mortality, decreased birth weight, decreased postnatal growth and/or viability, and structural malformation. Structural malformations will be referred to as terata. In this context, a chemical which is not teratogenic may be correctly referred to as a developmental toxicant. In contrast all teratogenic agents are, by definition, also developmental toxicants. Reproductive toxicology is defined as specific effects of toxicants on the reproductive organs. References to sperm morphology and male fertility would be refered to as reproductive toxicology. Effects on estrus would be referred to as reproductive toxicology. Teratology is an irreversible effect and, therefore, of special concern.

A summary of reports on developmental toxicity of carbaryl in mammals is presented for general reference asTable II-1. Tabulation is without regard to the validity of effect noted. When more than one study was available for a species the most complete was selected for summary in Table II-1. When more than one positive result was reported the study yielding the most significant positive effect was tabulated. Unless the claims of the author were clearly unsubstantiated by test data, the effect was accepted as reported. An obvious disadvantage of this approach is that it significantly over-emphasizes effects. Balance of evidence, validity of the results and impact on the overall evaluation of carbaryl are discussed in detail in the individual study reviews.

Data from the open scientific literature documents that under the right experimental conditions (*e.g.,* species, dose, route, time of exposure) carbaryl can produce developmental toxicity and, in some instances, teratogenicity. The significance of these findings for estimating risk to pregnant humans is influenced by several factors. The two most important of these factors are experimental design and dose. It is noteworthy that the conduct of the majority of studies describing potential developmental toxicity of carbaryl fall short of current guidelines. Shortcomings are readily apparent when comparing the implemented designs with the recommendations of Organization for Economic Cooperation and Development (OECD), the United States Food and Drug Administration (FDA) or the United States Environmental Protection Agency (EPA).

Expanded discussion of several studies is offered when possible teratogenic effects are reported. Doses resulting in maternal death were often necessary to produce developmental toxicity. Terata were only produced at exposures producing signs of maternal toxicity. The lowest NOEL was demonstrated as 2.0 mg/kg in dogs and represents approximately a 400,000- fold safety factor. Comprehensive evaluation of all the data available leads this reviewer to the conclusion that the overwhelming weight of evidence supports the following summary statement: *carbaryl does not pose an unreasonable risk to humans with regard to teratogenic potential when approved patterns of use are followed.*

TABLE II-1. Carbaryl Mammalian Developmental Toxicity Summary

Species	Effect							Noel[3]			
	Maternal[1] Toxicity	Fertility	Pre-natal Mortality	Birth Wt.	Post-natal Growth	Post-natal Surv.	Terata[2]	Diet (mg/kg/day)	Gavage (mg/kg/day)	Other (mg/kg/day)	Number of Studies
Rat	+[4]	+[5]	+	-	+	+	-	200	25	ND	11
Mouse	+	-	+	+	-	-	-	30	150	25	4
Gerbil	+	ND[6]	+	ND	+	+	ND	ND	ND	ND	1
Guinea Pig	+	ND	+	-	ND	ND	-[5]	200	200	ND	2
Hamster	+	ND	+	-	ND	ND	-	ND	125	ND	1
Rabbit	+	ND	+	-	ND	ND	+	30	150	ND	3
Sheep	-	-	-	-	ND	ND	?[8]	ND	ND	ND	1
Swine	-	+	S[7]	ND	ND	ND	-	ND	ND	ND	1
Dog	+	+	+	+	+	+	+	<2.0	ND	ND	2
Monkey	-	-	S	S	-	-	-	ND	20	ND	2

[1] Signs of maternal toxicity (*e.g.*, clinical toxicity, reduced weight gain, mortality) at doses also resulting in fetal toxicity.
[2] Structural Malformations
[3] No Observable Effect Level for developmental toxicity of any type. In some instances, assumptions were made concerning dietary intake inorder to arrive at total daily intake. See discussion in text.
[4] Adverse effects observed in at least one study.
[5] No adverse effects observed.
[6] Not determined, either by design of the study(s), or due to insufficient evidence for the endpoint.
[7] Suggestive, but inconclusive.
[8] Inadequate control for comparison.

The major experimental design deficiency in many of the available teratology studies was the small sample size of many of the experimental and control groups. Current guidelines recommend a minimum sample size of 20 pregnant rodents and 12 pregnant rabbits for conventional teratology studies, and a minimum of 20 pregnant rodents for standard reproduction bioassays. Group sample size is especially important when attempting to establish, with a reasonable degree of certainty, the absence of an effect. As sample size decreases, the power of the experiment, *i.e.*, the ability to detect real differences between groups, decreases. As sample size decreases the probability of a Type II error (*i.e.*, acceptance of the null hypothesis of equal means when it is false) increases, thus reducing the validity of "negative" results (Steel and Torrie, 1960). Studies with currently (or close to currently) recommended sample sizes include three of eleven rat studies (Collins *et al.*, 1971; Hart, 1972; Weil *et al.*, 1973), two of the four mouse studies (Benson *et al.*, 1967; Murray *et al.*, 1979), the single reported gerbil study (Collins *et al.*, 1971) and one of three rabbit studies (Murray *et al.*, 1979). Although no specific guidelines are available for large animal studies, the sample sizes in the one sheep study (Panciera, 1967), one of two dog studies (Imming *et al.*, 1969) and one of two monkey studies (Coulston *et al.*, 1974) were at least as large as recommended for rabbits. Studies conducted on the guinea pig, hamster and pig are insufficient for estimation of no observable effect levels (NOEL) by current standards, however they remain useful when evaluated with studies of more adequate size or as an element of a total data base.

Dose selection was the next most compromising departure from currently accepted practices for the conduct of developmental toxicity bioassays. Doses should be selected so that the high dose produces some form of maternal or fetal toxicity, while the low dose should produce no observable effect (NOEL). The rat study by Weil and Carpenter,

1966; the mouse study of Kotin *et al.,* 1968; the rabbit studies of Shaffer and Levy, 1968 and Robens, 1969; and the monkey studies of Dougherty *et al.*, 1971 and Coulston *et al.,* 1974 are of limited utility in the detection of possible adverse developmental effects of carbaryl due to a lack of either toxic or developmental effects at any dose. The results can be used to establish NOELs.

It is obligatory that developmental toxicity be evaluated with careful reference to effects observed in parental (particularly maternal) animals. If carbaryl causes developmental effects only at levels resulting in maternal toxicity, general toxicity rather than developmental toxicity has been demonstrated. This is an example of the generally accepted principle that any chemical, when administered at high enough doses to the right species at the right time of gestation, can produce developmental toxicity. This principle is important when attempting to utilize the results of the available studies to assess the potential developmental effects of carbaryl in humans. Examination of Table II-1 reveals that for all species except perhaps the sheep, maternal toxicity was observed at dose levels producing adverse developmental effects. When doses below those resulting in maternal toxicity were utilizied, no developmental toxicity was observed. Although, on a study-by-study basis, the significance of negative results (or no observable effect levels - NOELs) must be evaluated with caution, the extant data for the rat, mouse and rabbit establish, with a reasonable degree of certainty, the NOELs for these species. The sample sizes of the guinea pig and hamster studies are too small to support the apparent NOELs for these species however lower NOELs in other species eliminates any impact on the risk assessment process.

Evidence of developmental toxicity at doses not resulting in observable maternal toxicity was suggested for sheep (Panciera, 1967) where 2 of 23 offspring at the high dose diet group (250 ppm) were observed with cardiac anomalies, as compared to 0 of 44 animals at the low dose (100 ppm) and control animals. These data are not considered conclusive since the background incidence of anomalies in this species was not reported. Two studies in dogs (Imming *et al.,* 1969; Smalley *et al.,* 1968) demonstrated fetotoxic and teratogenic effects at doses producing evidence of maternal toxicity. Teratogenic effects were demonstrated at doses of 5.0 mg/kg/day and greater. The lowest dose utilized in the two studies (2.0 mg/kg/day) reduced postnatal viability although Imming *et al.* and Smalley *et al.* failed to report maternal toxicity *per se,* the doses utilized should have produced significant cholinesterase inhibition and did produce maternal toxicity as evident by the reported difficulties in parturition.

Some have suggested the presence of a unique metabolic pattern for the dog as compared to other species. Sullivan (1972) showed no major metabolites in agreement with humans and reported an anionic urinary metabolite uncommon to any other species studied. Several studies indicate similar metabolic patterns for carbaryl in the rat, guinea pig and human, with the guinea pig showing the best agreement with humans among the small animals studied. In contrast, results from the dog, sheep, pig and monkey are not in complete harmony with human data. These considerations lead the National Institute for Occupational Safety and Health (NIOSH) to conclude in 1976 that: "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" (Criteria for a Recommended Standard, 1976). Based on the evidence referenced above and no additional relevant data, it appears that such a conclusion is still warranted. Are there really important species differences in sensitivity to carbaryl or are the inconsistencies observed due in major part to variations in protocol? It is suggested by this reviewer that when cholinesterase inhibition is used as a comparative marker, species differences tend to disappear and that at doses permitting normal neuromuscular function in the mother, teratogenic effects are always absent.

A number of Soviet reports including those cited in the 1982 UNEP review of the Russian literature were carefully reviewed for relevance to the issue of carbaryl developmental toxicity. The majority of the reports were grossly deficient in generation,

presentation and/or analysis of data and those considered completely deficient are not addressed further. The most consistent effect reported was an altered gonadal histological structure and function in male and female rats exposed to carbaryl via gastric intubation. These effects included increased duration of the estrous cycle and impaired sperm performance. Shtenberg and Orlova (1970) demonstrated sperm functional parameters (e.g., sodium chloride resistance and mobility time) to be affected by simple dietary manipulations to a degree equaling or exceeding that of carbaryl administration. Vashakidze (1970) demonstrated high protein diets blocked the effects of carbaryl. The relevance of these data to evaluating risks after human dietary exposure is questionable at best.

The Soviet authors suggest that carbaryl administration to rats can produce alterations in gonadal structure and function, however, the data are inadequately documented and cannot as such be used in the determination of acceptable exposure levels in humans. In addition the studies reporting altered gonadal structure, estrus extension and sperm performance are not consistent with the broader body of more completely documented research, including studies on a human volunteer cohort.

Study Reviews by Species

Rat. Weil and Carpenter (1965) conducted a three generation, two litter study in CFE rats by administering carbaryl in the diet at levels necessary to maintain total daily intakes of 0, 2.5 or 10 mg/kg/day. Group sizes of 11-20 were utilized. No signs of maternal or reproductive toxicity were noted. The doses utilized were below currently accepted standards since no signs of maternal toxicity were reported.

Weil and Carpenter (1966) administered carbaryl in the diets of Harlan-Wistar rats on various intervals during gestation at dose levels of 0, 20, 100 or 500 mg/kg/day. Actual dietary concentration levels varied from approximately 300 ppm in the low dose to approximately 8600 ppm in the high dose group. One half (N=6) of the females in each dose level-treatment interval subgroup were sacrificed prior to expected parturition, with the other half allowed to litter and raise their offspring to weaning. The authors reported reduced maternal weight at 500 and 100 mg/kg/day. The only reproductive parameter significantly affected was reduced postnatal survival of pups in the 500 mg/kg/day group. The no observable effect level for fetotoxicity in this study was 100 mg/kg/day.

Dinerman *et al.* (1970) administered 0.02% of an LD_{50} of carbaryl by mouth to rats on days 9, 11, and 13 of gestation and produced only decrease in fetal weight .

Collins *et al.* (1971) administered carbaryl in the diet of three generations of male and female Osborne-Mendel rats at levels of 0, 2,000, 5,000 and 10,000 ppm. Two litters were produced from each generation. The fertility index (number pregnant/number mated) was significantly reduced in the second generation at 10,000 ppm, with no pregnancies observed in the second litter. Collins *et al.* (1971) reported reduced litter size at 5,000 and 10,000 ppm, reduced survival to day 4 at 5000 and 10,000 ppm, reduced survival from day 4 to weaning at 10,000 ppm, and reduced weaning weights in all treated groups compared to controls. Maternal toxicity, as evidenced by growth depression was observed at 10,000 ppm. Assuming an average daily food intake in female rats, of 75 gm/kg body weight, the total daily intake of carbaryl in this study would be 750, 375 and 150 mg/kg/day for the 10,000, 5,000 and 2,000 ppm groups, respectively. Controversial procedures for calculation of the reproductive indices were presented in this report. The data establish a no observable effect level of 2,000 ppm (or approximately 150 mg/kg/day).

Hart (1971) administered carbaryl in the diet to groups of 20 pregnant Sprague-Dawley rats on days 6-15 of gestation at levels of 0, 4,000 and 7,000 ppm (estimated total daily intake assuming 75 gm/kg/day food consumption = 0, 300, 525 mg/kg/day). The dams were sacrificed on day 18 of gestation. No adverse effects on fetal viability or

development were reported. Maternal food consumption and body weight was determined, but the data are not reported. The author stated, "The data on body weight gain on the part of the pregnant female and on their food consumption do not appear to show any evidence of maternal toxicity".

Weil *et al.* (1973) conducted a study designed to compare the effects of dietary and gavage routes of administration over a 3 generations and multiple litters in "Elias stock" rats. Total daily doses administered in the diet were 0, 7, 25, 100 and 200 mg/kg/day, while gavage doses were 0, 3, 7, 25 and 100 mg/kg/day. Signs of maternal toxicity, including decreased weight gain, clinically-observable cholinesterase inhibition and increased mortality, were observed in the 100 mg/kg/day gavage group. Signs of reproductive toxicity in this group included decreased fertility in production of the F_{1b} litters and decreased live birth litter size in the F_{2a} and F_{3a} generations. No teratogenic effects were noted in F_{3b} fetuses at any dose level. An increase in the median number of days after mating to birth of the first litter of the F_{1a}-F_{2a} generation was noted in the 200 mg/kg group. No observable effect level for fetotoxicity of 25 mg/kg/day by gavage administration, and 200 mg/kg/day by dietary administration can be demonstrated.

Pregnant rats exhale a higher proportion of ^{14}C activity from ^{14}C carbonyl-labeled carbaryl as carbon dioxide but excrete a smaller proportion of ^{14}C activity from ring-labeled carbaryl in urine (Strother and Wheeler, 1976). Carbaryl has been demonstrated repeatedly to cross the placenta, however, transplacental transfer is small. Ninety-six hours after oral administration of ^{14}C-methyl carbaryl to rats on the 18th day of pregnancy, only 0.3% of the administered radioactivity was found in the fetuses (Declume and Derache, 1976, 1977; Declume and Benard, 1977). While these findings may suggest that metabolism of carbaryl is increased during pregnancy it is cautioned that there is the unresolved possibility that some of the ring metabolites may be sequestered in the fetus.

Loehner and Abdel-Rahman (1984) administered carbaryl and malathion alone and in combination in the rat. Formulation grade carbaryl (0, 1, 10, and 100 mg/kg), formulation grade malathion (1 and 50 mg/kg), and a mixture of carbaryl/malathion (1/1 and 50/50 mg/kg) were administered daily by gavage for 3 months prior to and throughout gestation. Dams were sacrificed on day 20 of gestation, and the fetuses were examined for external, skeletal, and visceral malformations. Significant decreases in dam weight gain during pregnancy and a slight decrease in the number of implantations and number of live fetuses per dam were observed with the 100-mg/kg carbaryl group. Weights were further reduced in both combination doses. The combination dose groups showed a significant reduction in placenta weight. No increases were seen in skeletal or visceral anomalies for the individual treatment groups.

Rat - USSR Literature. Rybakova (1966) administered carbaryl for 12 months by gavage to "albino rats" at doses of 7, 14 and 70 mg/kg. Body weight was significantly reduced only in the 70 mg/kg group. The spermatozoa "motility period" decreased significantly at 6 months in the 14 and 70 mg/kg groups and for all groups at 12 months. Microscopic changes in the "structure of the seminiferous tubules" were reported in the 70 mg/kg group. The low dose (7 mg/kg) of carbaryl reportedly produced "focal edema of the interstitital tissue, desquamation of spermatogenic epithelium and destruction of the parenchyma".

Vashakidze (1970) administered oral doses of 2, 5, 15 or 50 mg/kg carbaryl for six months, or 100 mg/kg for 1 month to white rats (125 males and 125 females). The animals were further subdivided into 3 groups and administered either a high protein (22%) synthetic diet, low protein (9%) synthetic diet, or a standard rat chow containing 15-18% protein. Weight loss, most pronounced at the end of the experiment, was reported in the 50 and 100 mg/kg dose groups. Decreased resistance of sperm to a 1% sodium chloride solution was reported in the 5, 15, 50 and 100 mg/kg groups. "Spermatozoon mobility time" in the 5 and 15 mg/kg groups, and the "spermatozoon longevity" was significantly reduced in the 50 and 100 mg/kg groups. Feeding a high (22%) protein diet prevented

the functional changes in the sperm. These authors state in contrast to the reports of other authors "in the histological study of the testicles of the test animals in all of the test groups with the exception of those rats which had received carbaryl in a 100 mg/kg dose, no deviations were observed from those figures which had been obtained for the control group". No histochemical changes were observed in any of the treatment groups.

Shtenberg and Ozhovan (1971) administered either 2 or 5 mg/kg carbaryl to five generations of rats. Male and female animals in the generations II thru V were examined for reproductive function during treatment. In males, several of the spermatozoa functional tests frequently used by USSR scientists were altered in the treatment groups. Sperm activity (presumably percent motility) was not reduced until the fourth generation and then, only in the 5 mg/kg dose group. Other measures of sperm function such as resistance to a sodium chloride solution, time of motility, and "life in nutrient medium" were affected in both dose groups in various generations of animals. Alterations in the estrous cycle of the treated females were also reported in both dose groups. Details of the several tests were not available.

The only observation reported for female animals was a "tendency towards an increase in the average duration of the intra-estrus period in comparison with the control group" at 5 and 15 mg/kg. Vashakidze (1975) administered carbaryl, in doses of 1, 5, 10, 20, 40 and 50 mg/kg p.o. to male and female rats for 1 month. Using serum chemistry as the indicator, the author reported that the "dose of 20 mg/kg is the threshold dose with respect to the indicators of general toxicity". Effects on reproductive parameters were reported for the 1, 5, 10 and 20 mg/kg groups only. A number of histological and functional changes were reported in the testes of treated animals. These included the number of tubules containing cells in various stages of development, sperm counts, sperm motility time, and percent motility. With the exception of the number of tubules containing spermatogonia noted in the 1 mg/kg group, treatment related changes were only observed at 5 mg/kg or higher. When treated males were mated with untreated females, a decrease in the mean number of implantations and normal embryos was reported. The mean number of underdeveloped (undefined) and dead embryos per animal was also increased in all treatment groups, but not in a dose-response manner. In females, carbaryl in doses of 5 mg/kg and higher prolonged the diestrus period, while the estrus and metestrus were prolonged in the 10 and 20 mg/kg and 20 mg/kg groups, respectively. None of the estrus effects exhibited dose-response relationships. Mating trials of treated females with untreated males resulted in a decreased number of underdeveloped and dead embryos at 5 mg/kg carbaryl and higher. A decrease in the animals. The number of corpora lutea per female was reported in the treated (>5 mg/kg) lack of dose responsiveness of the data describing dead or underdeveloped embryos and estrus effects limits interpretation and suggests nonspecific stress due to treatment rather than carbaryl *per se.*

Mouse. Benson *et al.* (1967) administered carbaryl in the diet to groups of 20 mice at concentrations of 0, 67 and 200 ppm. Total targeted daily intakes in these groups were 0, 10 and 30 mg/kg, respectively. Treated diets were administered from gestation days 6-18. One half of the mothers were sacrificed on day 18, with the other half allowed to litter and raise their offspring for four days following birth. No adverse effects on fetal or neonatal development were noted. No signs of maternal toxicity were observed at either dose level. The utility of this study for examining the potential fetotoxic effects of carbaryl in the mouse is limited because of lack of maternally toxic dose.

Kotin *et al.* (1968) administered on gestational days 6-14, subcutaneous doses of carbaryl to several strains of mice at doses ranging from 25 to 464 mg/kg. Sample sizes varied from 6 to 18 per group. Reduced maternal weight gain and fetal weights were observed in several strains at doses down to 100 mg/kg. An increased incidence of anomalies was noted in the BL6 strain administered 100 mg/kg carbaryl in DMSO when compared to controls administered DMSO only. The sample size for this group was small

(N=6) and the results were not replicated in two subsequent trials. The results with respect to anomalies, therefore, should not be considered conclusive. A no observable effect level for developmental toxicity of 25 mg/kg/day was demonstrated.

Guthrie *et al.* (1971) examined the potential for production of resistant strains of mice to a single i.p. injection of carbaryl one week prior to mating. A number of common reproductive endpoints were reported in this study but the results were not detailed sufficiently to permit interpretation with respect to the potential of carbaryl to adversely effect reproduction in mice.

Carbaryl at dosages up to 34 mg/kg/day for 5 days to mice failed to affect prostate assimilation on testosterone, weight of the testes and sex glands, or (Thomas *et al.,* 1974), the ability to metabolize testosterone (Dieringer and Thomas, 1974).

There were no significant differences in population growth or genetic variation among feral mice in areas treated at the unusually high rate of 21.74 kg/hectare (ha) (Graf*et al.,* 1976). Carbaryl has been demonstrated to cross the placenta of mice (Declime and Benard, 1978).

Murray *et al.* (1979) administered carbaryl by gavage at levels of 100 or 150 mg/kg/day, or by diet at a level of 1,166 mg/kg/day (5660 ppm) on days 6-15 of gestation. Sample sizes were 20-34 pregnant animals. Gavage administration of 150 mg/kg/day was maternally toxic, as demonstrated by clinical signs of cholinesterase inhibition, mortality and depressed weight gain during treatment. At 100 mg/kg/day one maternal death was noted, but no other signs of maternal toxicity were observed. In the animals administered carbaryl in the diet, no deaths were noted, but depressed weight gain during treatment was observed. The only fetotoxic effects observed were decreased fetal weight and crown-rump length in the animals administered carbaryl in the diet. A significant increase in terata was not observed in this study.

Gerbil. Collins *et al.* (1971) administered carbaryl in the diet of three generations of gerbils at levels of 0, 2000, 4000, 6000 and 10,000 ppm. Reduced fertility was noted as low as 2000 ppm, although a clear dose-response trend was not observed. The authors reported a decrease in average litter size at all dose levels. These data are not, however, dose-responsive. Unorthodox methods for calculation of average litter size were used. A weak trend toward reduced litter size in the 10,000 ppm group was noted, however reduced fertility and neonatal survival resulted in too few litters for rigorous evaluation. Other trends from this study included reduced postnatal viability and weaning weights. The reduced postnatal survival (*i.e.,* survival to day 4) was dose-responsive, and is probably the most significant finding of the study.

Hamster. Robens (1969) administered carbaryl by oral gavage to groups of 6-8 hamsters during one to three day periods of organogenesis. The doses were 125 mg/kg administered on days 6-8 of gestation and 250 mg/kg administered either on day 7 or 8 of gestation. Concurrent vehicle controls were also utilized. Clinical signs of toxicity were noted in all treated animals with 2 of 6 animals dying in the 250 mg/kg groups. Increased fetal mortality was reported in the 250 mg/kg group.

Guinea Pig. Robens (1969) administered either single or multiple doses of carbaryl by gavage to groups of 5-26 guinea pigs at a level of 300 mg/kg. Not surprisingly significant maternal toxicity was observed in this study, with 10 of 26 animals dying when administered the chemical on gestation days 11-20. Even single-day administration resulted in deaths in some animals. Increased fetal mortality was observed in the animals treated on gestation days 11-20, but not in those dosed on single days. Skeletal anomalies, primarily of the cervical vertebrae were noted in animals treated with both single and multiple doses of carbaryl. While the total number of fetuses with terata are presented, the distribution of the fetuses among litters is not. These data demonstrate that at maternally toxic/lethal dose levels carbaryl is fetotoxic and, in some instances teratogenic in the guinea pig.

Weil *et al.* (1973) examined the effects of dietary and gavage administration of carbaryl during various intervals of gestation. Doses utilized in the dietary study were 0, 100, 200 and 300 mg/kg/day. Gavage doses were 0, 50, 100 and 200 mg/kg. The study was further subdivided by the number of doses and the interval of administration. Sample sizes were 5-10 per subgroup. Preliminary studies revealed clinical signs of cholinesterase inhibition at all gavage dose levels and depressed weight gains in animals fed carbaryl in the diet at a level of 300 mg/kg. The only statistically significant fetotoxic effect noted in this study was a reduced number of live fetuses per litter in the animals fed single doses of 300 mg/kg of carbaryl in the diet during the interval 15-19 days of gestation. No significant increases in skeletal or visceral anomalies were noted. The significance of these findings are difficult, if not impossible, to assess due to pooling of data in the report.

Rabbit. Shaffer and Levy (1968) administered total daily doses of 0, 10 and 30 mg/kg/day of carbaryl in the diet on gestation days 9-16. A total of 15 adult females were inseminated and dosed at each level, but the number that produced viable litters was only 7, 7 and 5 for the 0, 10 and 30 mg/kg/day groups, respectively. No signs of maternal or fetal toxicity attributable to carbaryl administration were noted at any of the dose levels in this study. Due to the low dose levels and sample sizes utilized, the results of this study are not meaningful in regards to assessment of the fetotoxic effects of carbaryl.

Robens (1969) administered carbaryl by oral gavage to groups of 4-9 rabbits at levels of 50, 100 and 200 mg/kg/day on gestation days 5-15. A vehicle control group of 21 rabbits was utilized. All animals were sacrificed on day 28 of gestation. No maternal or fetotoxic effects were reported in this study.

Murray *et al.* (1979) administered groups of 13-20 rabbits 0, 150 or 200 mg/kg/day of carbaryl by gavage on days 6-18 of gestation. Diarrhea was observed at 200, but not 150 mg/kg/day. Decreased weight gain during treatment was noted in both groups as compared to concurrent controls. The only evidence of fetal toxicity was a trend towards an increased incidence in fetal resorptions in the 200 mg/kg/day group, and a significantly reduced fetal weight in the 150 mg/kg/day group. The fetal weight reduction should not be considered a compound-related effect, since it was not observed in the 200 mg/kg/day animals and, since the actual value was within the range for previous control groups in the same laboratory. A significant increase in terata (omphalocele) was noted in the 200, but not the 150 mg/kg/day animals.

Sheep. Panciera (1967) fed carbaryl to groups of 22-23 Ramboillet sheep during breeding and gestation at levels of 0, 100 and 250 ppm. No clinical signs of toxicity were noted during the study. Cardiac anomalies (VSD) were observed in 2/23 lambs in the 250 ppm group. No such anomalies were noted in the 100 ppm or control groups. No statistical evaluation of the data were performed.

Swine. Earl *et al.* (1973) administered carbaryl in the diets of miniature swine at levels of 0, 4, 8, 16 and 32 mg/kg/day. Samples sizes of 5-16 were utilized. The data were not statistically analyzed, and the only apparent trends in the study were a reduction in fertility and number of pigs per litter in the treated animals. These data, however, are not sufficient to definitely establish fetotoxic effects (or the lack thereof) in this species.

Dog. Imming *et al.* (1969) incorporated carbaryl into the diet of groups of 12 beagles at levels of 0, 2.0, 5.0 and 12.5 mg/kg/day. Administration continued from day 1 of gestation until weaning of the offspring. Results included increased stillbirths at 5 and 12.5 mg/kg/day, decreased birth weight at 12.5 mg/kg/day and decreased postnatal survival at all dose levels. For the latter effect (postnatal survival), weaning mortality was 14%, 40%, 41% and 41% in the control, 2.0, 5.0 and 12.5 mg/kg/day dose groups. Terata were observed in 3 of 9 litters at 12.5 mg/kg/day, and 5.0 mg/kg/day. No terata were observed in the control or 2.0 mg/kg/day animals.

Smalley *et al.* (1968) and Earl *et al.* (1973) administered carbaryl in the diet to groups of 2-16 beagles at dose levels of 0, 3.125, 6.25, 12.5, 25 and 50 mg/kg/day. Findings included a dose-responsive decrease in litter size and postnatal viability, increased prenatal mortality, decreased postnatal growth, and dystocia (difficult labor) in all groups. Terata were observed at doses of 6.25 mg/kg/day and higher. Tables II-2 and II-3 present the data of Smalley *et al* (1968) and Earl *et al* (1973) for comparison of pre and post natal toxicity.

Nine implantations per litter were expected. Six implantations were observed at 50, 25 and 12.5 mg/kg/day carbaryl. Therefore, carbaryl does exhibit maternally mediated preimplantation toxicity. No preimplantation toxicity was observed at 6.25 or 3.125 mg/kg/day carbaryl.

When the data are presented by adjusting for the maternally toxic preimplantation effect and resorptions there is no difference between groups in animals successfully weaned. In other words, the effects observed are due to toxic effects on the mother. The maternal toxicity is responsibile for effects on the embryo and early fetus as demonstrated by resorptions; on the late fetus, as demonstrated by late fetal death and; on the weanling as demonstrated by dystocia and failure to survive weaning. The phenomena can be described as toxicity which is expressed at earlier stages of development as the dose increases.

The terata observed in the dog are most likely due to nonspecific maternal toxicity rather than specific teratogenic mechanisms. This statement is supported by several major observations.

1. A variety of embryologically unrelated terata were produced.
2. The malformations are for the most part "weak links" in highly inbred dogs and expressed in the presence of stresses such as administering toxic levels of carbaryl.
3. No dose response is demonstrated.
4. All doses with the possible exception of 3.125 mg/kg/day produced toxicity to the embryo, fetus, neonate or mother .
5. All dose groups producing live pups yielded identical percentages of implants surviving weaning.

TABLE II-2. Effects of Carbaryl on Prenatal Toxicity in Dogs.[a]

Dose (mg/kg)	Conception Rate	Implantations per Litter	Resorptions per Litter	Resorptions per Implantation	% Fetal Death	% Dystocia Rate
50	37	6.0	2.5	0.42	100	100
25	78	6.5	2.7	0.41	40	33
12.5	89	6.1	1.2	0.20[b]	62	28
6.25[c]	80	9.6	4.7	0.49	38	30
3.125[c]	70	8.7	3.1	0.36	34	30
0[d]	81	-	-	-	19	0
0[d]	80.25	-	-	-	14	0

[a]Rev: Smalley *et al, 1968* ; Earl *et al,* 1973; Imming, 1969.
[b]12.5 mg/kg/day group is different than all other treatment groups in that it is the only group without a female yielding 100% resorptions.
[c]No preimplantation toxicity observed.
[d]Control

Several points support the argument that 3.125 mg/kg/day is a NOEL for malformations. First, the number of pups per litter are the same as the controls; next,

there were no abnormal litters or pups, and that the expected number of pups born alive survived weaning.

A NOEL for fetal toxicity was not observed since there was a doubling of death at the lowest dose, 3.125 mg/kg/day carbaryl utilized by Smalley *et al.* (1968) or 2.0 mg/kg/day by Imming *et al.* (1969).

A significant departure from standard teratology protocol was implemented in both dog studies when carbaryl was administered from day 1 of gestation throughout weaning. This procedure compromised the uterine environment prior to implantation, and did does not permit the female to recover prior to the birthing trial, and continues to spread to the neonate.

TABLE II-3. Effects of Carbaryl on Postnatal Toxicity in Dogs.[a]

Dose (mg/kg)	% Abnormal Litters	% Pups Abnormal	Pups per Implantation	Live Pups per Implantation	%Pups Weaned	%Pups Weaned per Implantation
50	50	14	0.58	0	NA*	0
25	50	13	0.59	0.24	65	16[c]
12.5	19	18	0.80	0.50	61	30[c]
6.25	14	9	0.51	0.19	81	15[c]
3.125	0	0	0.64	0.22	91	18[c]
0[d]	0	0	NA[b]	NA[b]	90	NA[b]
0[d]	0.9	0.1	NA[b]	NA[b]	95	NA[b]

[a]Ref: Smalley *et al*, 1968; Earl *et al*, 1973; Imming, 1969).
[b]Not applicable since no pups born alive.
[c]No difference in groups producing live pups at birth.
[d]Control

Monkey. Dougherty *et al.* (1971) administered carbaryl to groups of 2-6 Rhesus monkeys by gavage at dose levels of 0, 2.0 and 20 mg/kg/day throughout gestation. The number of animals delivering viable offspring were 3/6 in the control, 0/2 in the 2.0 mg/kg and 3/6 in the 20 mg/kg groups. A greater number of abortions occurred in the treatment groups (5/8) as compared to controls (1/5), but the validity of this finding is dependent on the accuracy of the pregnancy test utilized. No adverse effects were noted in any of the offspring in this study.

In a more definitive and controlled study Coulston *et al.* (1974) again administered carbaryl by gavage to Rhesus monkeys. The groups (N=16) included untreated control, vehicle control and carbaryl dose groups of 0.2, 2.0 and 20 mg/kg/day administered on gestation days 20-38. Mothers were allowed to deliver, and offspring were examined for up to one year following birth. No signs of maternal toxicity were noted. Two offspring in the 20 mg/kg group had low birth weights but no other signs of fetotoxicity were noted in this study.

Chicken. Only 0.0008% of an oral LD_{50} caused 100% mortality when injected into chcken embryos. These findings are of little to no consequence since Khmelevskii and Stephanov (1969) found that chicks developed normally even when carbaryl was fed to hens and cocks at a level that did not lead to overt illness but reduced their blood cholinesterase and led to residues in both tissues and eggs (0.02 to 0.06 ppm). In addition Lillie (1973) found that dietary levels up to 500 ppm, which caused significant growth depression in the adult, did not interfere with egg production, fertility, or hatchability, and there was no embryonic abnormality.

Swartz (1985) explored the effect of carbaryl during the period of primordial germ cell migration and mid-way in embryonic development following sex differentiation in the chick embryo. Any alteration in the migration of these cells and subsequent colonization of the gonads would seriously hamper or eliminate the reproductive activity of these animals. Fertile white Leghorn eggs were randomly divided into two groups with one receiving 10 mg carbaryl by injection (technical grade, 99%) dissolved in acetone and the other the acetone vehicle only. Eggs were removed from the incubator after 5 days (Stages 24-27) and at 12 days of incubation (Stages 38-39). Embryos were removed, fixed, dehydrated, embedded, serially sectioned, and stained. The primordial germ cells of 5 day embyos located both in the gonads and adjacent dorsal mesentery were counted. Mitotic activity of the primordial germ cells was also recorded. The number of primoridal germ cells found in the embryos exposed to 10 mg of carbaryl for 5 days was not significantly ($P>0.05$) different than controls and histology of the gonads and primordial germ cells was not altered. The mean number of primordial germ cells in the gonadal area was lower than that of controls, in embryos exposed to carbaryl for 5 days, but this difference was not statistically significant. Embryos exposed to the pre-incubation injection of carbaryl for 12 days survived the exposure very well, and exhibited no alterations in testicular or ovarian morphology or sex ratio. Histologically, the gonads from treated embryos differed little from those of control embryo gonads. Data from this study indicate that carbaryl does not produce deleterious effects on the developing avian gonads.

Conclusions

Carbaryl, when administered by various routes, at doses toxic to the maternal animal, has been shown to produce developmental toxicity in a number of species, including rats, mice, gerbils, hamsters, rabbits, pigs and dogs. Frank terata have been demonstrated only at levels causing cholinesterase inhibition and other indicators of maternal toxicity. In 1973, the World Health Organization (WHO) recommended Acceptable Daily Intake (ADI) of carbaryl of 0.01 mg/kg/day in humans. The lowest NOEL obtained from the studies in dogs was 2 mg/kg. When the 2 mg/kg NOEL is compared to the 0.01 mg/kg ADI a 2,000 fold safety margin is revealed. Actual carbaryl exposures are more likely .000005 mg/kg/day (See VI: Human Exposure) providing a 400,000 fold safety factor. It is concluded by this reviewer that carbaryl does not pose any risk, certainly no unreasonable risk, to the pregnant human or her offspring when exposure is at those levels anticipated for the general population under approved patterns of use.

III: MUTAGENICITY

Summary

The purpose of testing for mutagenicity is to determine the potential to cause heritable effects in man. A functional human mutagen must be capable of reaching human germinal tissues with the capability of irreversibly damaging the DNA of germ cells but only to the extent which still permits damaged cells to survive and produce viable progeny. All the requisite steps are necessary before a potential heritable effect in humans can be produced. The US-EPA (1980) concluded that the evidence available did not support the presumption that carbaryl posed a risk of inducing changes in the genetic complement of either somatic or germinal tissue in of humans. EPA (1980) stated that they did not reach their conclusion on the basis of any given test but on the totality of the evidence available attempted to balance the quality of work, the appropriateness of the test and the relevance of other data. EPA noted, as have other

reviewers, that a chemical which is positive in one sensitive screening *in vitro* test may be negative in another.

The data base is larger but not significantly different than in 1980 when EPA offered their conclusion. The weight of evidence remains the same. Carbaryl, at toxic doses, can produce disruption of or changes in the genetic apparatus of organisms used as sensitive screening tests but is unlikely to represent a risk to humans. The balance of evidence supports the conclusion that carbaryl and its possible *in vivo* metabolites, including N-nitrosocarbaryl, do not represent a quantifiable mutagenic risk to humans.

Mutagenicity - Carbaryl

Bacterial Test Systems. Carbaryl has been tested in a number of bacterial assay systems which measure genetic alterations in the form of specific locus gene mutations. The most frequently used *in vitro* system is the Ames Salmonella/Microsome assay. The system detects reverse mutations in a series of histidine-requiring auxotrophs of *Salmonella typhimurium* (Ames, *et al.,* 1975). The endpoint is detected as a heritable change in phenotype (ability to grow in the absence of exogenous histidine) caused by a reverse mutation occurring at the histidine locus. Incorporation of microsomal metabolic activation (S-9) systems into the standard assays increases sensitivity and relevance as predictors of the genotoxicity of compounds requiring bioactivation. Most point mutation assays measure either forward or reverse mutation in bacteria and duplicate the detection pattern of the Ames assay.

McCann *et al.,* 1975; Shirasu *et al.,*1976; Marshal *et al.*, 1976; Egert and Greim, 1976; Blevins *et al.,* 1977; Cook *et al.,*1977; Rashid, 1978; De Lorenzo *et al.,* 1978; Jaszozuk *et al.,* 1979; and Moriya *et al.,* 1983, have published on the mutagenic activity of carbaryl. These reports have included tests with and without metabolic activation and have included Salmonella strains TA-1535, TA-1536, TA-1537, TA-1538, TA-98 and TA-100. Test levels as high as 1,000 µg/plate have been used.

EPA (1984) provides guidance in determining if Ames test results indicate mutagenicity. A result in the *Salmonella* system is positive if there is a statistically significant dose-related increase in the number of revertants or the detection of a reproducible and statistically significant positive response for at least one of the concentrations tested. A negative response is recorded if the test does not produce either a statistically significant dose-related increase in the number of revertants or a statistically significant and reproducible positive response at any one of the concentrations tested. All *Salmonella* tests of carbaryl reviewed herein are negative by EPA criteria.

Ashwood-Smith *et al.,* 1972; DeGiovanni-Donnelly *et al.,* 1968; Egert and Greim, 1976; Elespuru *et al.,* 1974; Fahrig, 1974; Ficsor and Nil Lo Piccolo, 1972; Nagy *et al.,* 1975 and Shirasu *et al.,* 1976; used several other bacteria systems to test for forward and reverse mutations. None of these tests were positive.

Carbaryl does not induce gene mutations in bacterial systems.

Mammalian Cell Culture . The effect of carbaryl on the induction of ouabain-resistant mutants in Chinese hamster V-79 cells at concentrations of 10 µM was reported by Ahmed *et al.* (1977a). The mutation frequency induced with carbaryl at a 10 µM concentration was 15.3 per 10^6 survivors, about a 9 fold enhancement. In the same test chlordane, dieldrin and 2,4- dichlorophenoxyacetic acid produced mutation frequencies of 26.9, 16.4 and 25.5, respectively. The investigators concluded that carbaryl enhanced the number of ouabain-resistant mutants and acted as a weak mutagen. Several problems compromised with the test including; a 34% lethality at the test dose; no dose response. All chemicals tested, although diverse in structure, yielded approximately the same result. Data were not presented to support statements of phenotypic stability and the study has not been repeated.

Wojciechowski *et al.,* (1982) also studied the potential for induction of ouabain resistance in V-79 cells by carbaryl. Carbaryl did not affect the mutation frequency either in the presence or absence of metabolic activation.

The data for V-79 cell culture are mixed but on balance judged negative by this reviewer.

DNA Damage. Mammalian cells normally synthesize DNA during a single stage of the cells cycle referred to as scheduled DNA synthesis. When DNA is damaged unscheduled DNA synthesis can occur. A test system which quantitatively measures DNA repair can be an indicator of previous or simultaneous primary DNA damage. Since all normal organisms are capable of some type of DNA repair, a significant increase in the level of repair activity following chemical treatment is reasonably considered a general indicator that the test chemical may have been genotoxic. The reader is cautioned to remember that these tests do not measure mutations, they measure the relative activity of DNA repair. DNA repair is only assumed to reflect agent induced damage to DNA. Alternative explanations include disruption of cellular cycle, inactivation of repressor genes and stimulation of or preparation for cellular reproduction.

A single positive report suggests carbaryl damages DNA (Ahmed *et al.* 1977b). The results lead this reviewer to the conclusion that DNA repair can be stimulated by factors other than primary DNA damage. This viewpoint is supported by reviewing other work of Ahmed *et al.* (1977a).

Ahmed *et al.* (1977b) conducted tests to determine; if agents tested could induce unscheduled DNA synthesis (excision repair); if there was an effect of metabolic activation; if a dose effect could be produced and; the size of any repaired regions. SV-40 transformed human fibroblast cell line VA-4 was used throughout. Ahmed *et al.* (1977b) has been widely quoted as providing data supporting the mutagenicity of carbaryl. Therefore, a more in depth than usual analysis of the appropriateness of the test is provided.

Data from the study indicated that only one of the twenty-six tests conducted on thirteen separate compounds (lindane without metabolic activation) was without activity. The text states that ten compounds had significant activity. The test failed to distinguish between compounds with and without mutagenic and carcinogenic activity. It also failed to discriminate between compounds of vastly different structures and presence or absence of mutagenic/carcinogenic capabilities.

Metabolic activation enhanced the activity of heptachlor, heptachlorepoxide and dimethoate; diminished chlordane activity, and had no effect on carbaryl and eight other compounds.

No quantitative dose response data were offered. Carbaryl enhanced unscheduled DNA synthesis by eight times background at 100 μM. This effect was about 50 times less, on an equimolar basis, than that obtained for chlordane, dieldrin and 2,4-D fluid.

Carbaryl when administered 1, 10 and 100 μM induced 0.5, 0.6 and 1.5 breaks per 10^8 daltons of DNA. It should be noted that in studies by Ahmed *et al.* (1977a) and Ishidate and Odashima (1977) demonstrated that 100 μM carbaryl is extremely toxic to Chinese hamster fibroblasts. Chlordane, dieldrin and 2,4-D fluid were also tested and yielded similar results e.g., no dose response. No statistical analysis to support the observation of a positive effect at any dose was presented. No control data were offered.

Ahmed *et al.* (1977b) stated that the removal of "chemically bound DNA regions is saturated at relatively low concentrations" (1 and 10 μM). If this were true it would follow that the increased activity obtained with carbaryl at 100 μM is probably not due to carbaryl bound DNA since 1 and 10 μM would have been expected to have already saturated the DNA eliminating the opportunity for incremental response.

The authors stated that "at similar concentrations these insecticides are approximately 40% and 100% as effective as N-acetoxy-AAF and ICR-170 (UV-type) respectively in inducing BUdr containing regions in human fibroblasts and that the herbicide is

approximately twice as effective as the chemical alkylators MMS and propane sulfone for the induction of x-ray type repair in human fibroblast tissue". N-acetoxy-AAF, ICR-170, MMS and propane sulfone are all potent mutagens and carcinogens yet in the tests of Ahmed *et al.* (1977b) yield the same results as the poorly reactive representatives of several diverse classes of chemicals. The test clearly does not discriminate on the basis of ability to alkylate DNA and therefore must be producing the observed response through other toxic mechanisms. Support for such a possibility was reported by Ishidate and Odashima (1977) who detected aberrant chromosomes in 24% of cells exposed to 75 µM carbaryl but none at 37.5 µM.

The tests of Ahmed *et al.* (1977b), are interesting, but fail to meet the requirements of measuring primary damage to DNA via mechanisms in ways relevant to determining mutagenic risks.

Other work pertinent to this discussion has been performed by Regan *et al.* (1976) who found no evidence of DNA damage when they treated cultured human skin cells with 100 µM of carbaryl for 1 hour and determined sedimentation profiles of cellular DNA in alkaline sucrose gradients.

Siebert and Eisenbrand (1974) used a diploid strain of *Saccharomyces cerevisiae* D4 heteroallelic at the gene loci ade-2 and try-5 to assay for the ability of carbaryl to induce mitotic gene conversion in these loci. This is another assay that detects damage to DNA. In this organism, genetic activity (genetic damage) was not produced by a 16-hour carbaryl (1,000 ppm) treatment.

Carbaryl has not been shown to produce dose response related primary DNA damage.

Chromosomal Damage: *In Vitro*. Genetic damage can be in the form of microlesions, in the form of point mutations, as detected by the bacterial tests previously discussed or macrolesions, such as a chromosomal mutations. Chromosomal mutations include the gain, loss or rearrangement of segments and the addition or deletion of intact chromosomes. Chromosomal abnormalities can be detected visually. Cytogenetic studies designed to detect colchicine mitosis, chromosome lagging, chromosome fragmentation, multipolar anaphases, anaphase bridges, multinucleated cells and mitotic disturbances such as interfering with the spindle mechanism.

Carbaryl, at toxic doses, is capable of breaking chromosomes of plant cells in culture predominantly by interfering with spindle mechanism. (Wuu and Grant, 1966; Amer, 1965; Amer *et al.*, 1971; Amer and Farah, 1968; Brankovan, 1972).

Several Russian workers have reported that carbaryl may be an antimitotic agent in human and rat cells in culture at toxic doses (Shpirt, 1975; Kazarnovskaya and Vasilos, 1977; Vasilos *et al.*, 1972, 1975).

Vasilos *et al.* (1972) determined that activity was proportional to concentration and incubation time when human embryonic fibroblasts were exposed to 20, 40, and 80 ppm of carbaryl in the culture medium. Mitotic activity was totally suppressed by 80 ppm after 48 hours. Growth of HeLa cells is inhibited at much lower concentrations (4 and 8 ppm), but cell division is stimulated by 1 and 2 ppm (Blevins and Dunn, 1975).

Kazarnovskaya and Valsilos(1977) investigated the cytogenetic activity of carbaryl on a primary culture of human embryonic fibroblasts. Cells were treated with 20, 40 and 80 µg/ml of carbaryl in the growth medium. The 20 µg/ml dose caused no visible cell toxicity while the 80 µg/ml dose killed approximately 50% of the cells. Results indicated that the number of cells with chromosomal aberrations and the number of damaged chromosomes increased as the dose of the test chemical increased. The most frequent type of aberration was single fragments. The numbers of paired fragments as well as chromatid and chromosomal exchanges increased significantly only at the 80 µg/ml level. Aberrations such as asymmetric exchanges, ring chromosomes, pericentric inversions and exchanges between ends were not observed in either the control or treated groups. Carbaryl did not appear to cause selective damage of individual chromosomes but did

cause a statistically significant increase in the number of cells with altered chromosomal coiling (9.8% control, 17.9% at 8 µg/ml) and aneuploidy (3% control, 29.2% at 80 µg/ml).

Ishidate and Odashima (1977) studied the effects of carbaryl on chromosomes of cultured Chinese hamster fibroblasts. Doses of 7.5, 15 and 30 µg/ml were utilized. At the maximum effective dose, 30 µg/ml (50% growth inhibition dose), several types of chromosome aberrations (35% aberrant cells) were reported present 48 hours after treatment. Specifically, chromatid gaps and breaks, chromosome breaks, translocation, ring formation, and fragmentation were observed with a higher frequency in treated than in non-treated control cultures (1% aberrant cells). Although the authors stated the "gaps" were the predominant chromosomal effect, the frequency of occurrence for each particular type of aberration and the frequency of aberrations within a cell were not given. The 15 µg/ml level resulted in 24% aberrant cells while 7.5 µg/ml did not appreciably affect chromosome structure (1% aberrant cells). The authors did not provide data on the toxicity to the fibroblasts at either of the lower doses. The dose response was steep and a threshold was suggested.

The relative importance to be assigned to chromosomal mutations observed in cell cultures, for predicting *in vivo* effects and extrapolation, is difficult to ascertain. Antimitotic activity has been ascribed by the work on many workers in diverse system to most if not all pesticides investigated, even though they differ markedly in structure and pesticidal activity (insecticides, fungicides, herbicides etc.) (Shpirt, 1973). Spindle poisoning by pesticides has been reviewed (Hayes, 1975).

Brzheskii (1972) studied the mutagenic properties of carbaryl in *Drosophila melanogaster* utilizing a normal line from the Moscow, Russia area which had historically exhibited a low mutation rate and a laboratory line with linked X chromosomes homozygous for three recessive genes and line f. The test was designed to detect deletions in the X chromosome, recessive sex linked lethal and sublethal mutations and the fetility of males in successive matings. An LD_{50} feeding stock of a 85% carbaryl wettable powder as a 1% suspension in dilute sugar syrup was fed the males for 24 hours. A total of 7,089 offspring were examined. No deletions or infertility were detected.

Brzheskii (1972) also examined 2,747 control and 5,482 treated chromosomes of *D. melanogaster*. A confounding factor for this study was that a control mutation frequency of 0.05 ± 0.02% was expected on the basis of historical data, but not found. The frequency of mutations arising in the carbaryl treated group was not different than control at individual stages of spermatogenesis. A mutation rate of 0.20 ± 0.07% was recorded. When all stages of spermatogenesis were combined the treated group was different than the concurrent control. The author concluded that carbaryl did not induce deletions or change fertility but did increase recessive sex-linked lethals.

Carbaryl was tested by Woodruff *et al.*, (1983) for the ability to induce complete and partial chromosome loss in screens with MUS-302 repair-defective females of *D. melanogaster*. The rationale supporting the utility of this test is the ability of maternal enzymes in the zygotes to repair genetic damage in sperm . The results were negative for carbaryl.

A study by Hoque (1972) using wild type *D. melanogaster* reported that when females were fed carbaryl at 1, 5 and 10 ppm for 5 hours and then allowed to mate, both phenotypic and chromosomal abnormalities are observed in the F_1 and F_2 generations. More males than females were produced. Phenotypic abnormalities included the presence of a significant number of black-eyed and white-eyed F_1 and F_2 progeny from treated flies while controls produced only the red-eyed wild type offspring. Chromosomal abnormalities such as deficiencies, duplications, inversions and reciprocal translocations were observed. Low frequencies (often 1/treatment group), three times as many treated animals examined as controls, inconsistent and inverted dose response relationships, no statistics, and the small size of each group (some less than 40) casts doubts about the significance of the data.

Carbaryl is capable of breaking chromosomes *in vitro* in cell culture at highly toxic doses and *in vivo* in insects. Dose response slopes are steep and thresholds are suggested. Non specific toxic responses due to insecticidal properties of carbaryl may have produced a selective pressure for certain phenotypes in *in vivo* tests with insects. Carbaryl does not represent a mutagenic risk except at doses producing some lethal effects in the test systems.

Chromosomal Damage - *In Vivo.* Each of fourteen generations of mice were injected intraperitoneally with a single LD_{50} dosage of carbaryl at 6 to 8 weeks of age. LD_{50} determinations made at the fifth, tenth, and fifteenth generation were in general agreement with the LD_{50} measured before the study was started. No trend was detected. Variables associated with growth and reproduction were unaffected by the selection due to lethal effects. The untreated terminal generation was tested for a number of physiological and biochemical variables. No significant change in any variable was reported (Guthrie *et al.,* 1971).

Carbaryl has been tested for mutagenic activity in mice using the dominant lethal assay. The genetic basis for dominant lethality is primarily the induction of structural and numerical chromosomal abnormalities, such as translocations and aneuploidies. These abnormalities could induce preimplantation losses of nonviable zygotes, early fetal deaths and sterility or decreased fertility in F_1 progeny. The parameters examined in the dominant lethal test are usually percent pregnancy and total implants per pregnancy which consist of living implants and early fetal deaths (late fetal deaths are rare in these experiments).

Weil and Carpenter (1965) reported the results of a 3 generation reproduction study on rats fed 0.0, 2.5 and 10 mg/kg body weight. From 12 to 20 females per dosage level were bred in each of three generations. All criteria summarized, *i.e.,* total pups born, those born alive, those born dead and those weaned as well as fertility, gestation, viability and lactation indices were statistically similar between treated and control.

A dominant lethal assay was conducted by Epstein *et al.* (1972) who administered to mice100 mg/kg and 50 mg/kg of carbaryl by gavage equally over five consecutive days. Significant early fetal deaths or preimplementation losses were not produced.

Usha Rani *et al.* (1980) studied carbaryl in mice via the micronucleus test. Carbaryl at 146 mg/kg was administered orally in distilled water in two doses separated by 24 hours. No significant effect on the frequency of the micronucleus test was detected.

A host-mediated assay was performed following administration of carbaryl in three equal doses over 3 days. One day after the last dose the mice were injected ip with *Salmonella typhimurium* G-46 (Fahrig, 1974). No increase in mutation rate was observed.

Whorton *et al.* (1979) examined semen samples from 47 carbaryl production workers with at least one year of experience. A group of chemical plant workers, not previously exposed to carbaryl served as an external control. The authors found no association between sperm count levels and either the degree or duration of exposure to carbaryl. There was a small excess in the number of individuals with oligospermia (<20 million sperm/ml ejaculate) in the carbaryl workers, but the excess was not statistically significant. Reproductive hormone levels in the carbaryl exposed workers were normal.

Paradoxically when the semen samples collected by Whorton *et al.* (1979) were examined for abnormal sperm morphologies, an elevation of sperm abnormalities (abnormal head morphology) in *currently* exposed workers was reported (Wyrobeck *et al.,* 1980), but *previously* exposed workers did not exhibit significantly increased sperm anomalies. Furthermore, when duplicate slides to those examined by Wyrobeck *et al.* (1980) were examined via blind code by an independent examiner (MacLeod, 1982) the following conclusions were obtained: 1) there was not an obvious depression of the sperm count in the exposed group; 2) the pattern of sperm morphology in both the treated and control group was "excellent"; and 3) there was no difference in the distribution of the sperm types. Statistical analyses of these findings confirmed the

authors impressions (Hansen, 1982). Certain of the study results, including a lack of correlation between the degree of exposure and the incidence of abnormalities; a negative correlation between years of exposure and the percentage of abnormal sperm and the finding of no abnormalities in the exposed group suggest that there is no link between carbaryl exposure and human seminal defects. Similar conclusions to those presented above were expressed by the US EPA (1980) in a comment on the gonadal effects of carbaryl.

Carbaryl is negative when tested for mutagenicity in mammlian systems and is without effect in humans occupationally exposed.

Mutagenicity - N-nitrosocarbaryl

Siebert and Eisenbrand (1974) tested the potential of N-nitrosocarbaryl to induce mitotic gene conversions in *Saccharomyces cerevisiae*. This system measures the induction of mitotic gene conversion (intragenic recombination) at two different heteroallelic loci, adenine (ade 2) and tryptophan (try 5) in the diploid yeast. The amount of induction can be interpreted as an index of the genetic damage produced by a test chemical. N-nitrosocarbaryl was found to be very active in this system. Exposure for 2 hours in 0.21 mM solution resulted in about 74% killing and the relative conversion frequencies were increased by 139-fold for the ade 2 locus and 885-fold for the try 5 locus. Nontoxic concentrations of 0.004 mM and 0.04 mM resulted in significant results. The relative conversion frequency increased rapidly compared to the control from three fold (ade 2) and five fold (try 5) in the 0.004 mM solution to about 52 fold (ade 2) and 75 fold (try 5) in the 0.04 mM concentration. A dose related effect was shown over five concentrations of N-nitrosocarbaryl.

Elespuru, *et al.* (1974) measured the mutagenic activity of N-nitrosocarbaryl in *Escherichia coli* strain H/r 30 R, an arginine auxotraph. At a concentration of 0.1 mM, carbaryl killed approximately 50% of the cells and induced a mutation frequency (reversion to prototropy) of 3×10^{-4} per survivor compared to a background of 2×10^{-8} per survivor. N-nitrosocarbaryl was reported as more potent than MNNG. These same investigators compared mutagenic activity in the *Haemophilus influenzae* system which measures the induction of resistance to the antibiotic novobiocin. N-nitrosocarbaryl was more potent than MNNG and less toxic and more mutagenic with *Haemophilus influenza* than with *E. coli.*

Uchiyama, *et al.*, (1975) examined the mutagenic potency of N-nitrosocarbaryl using the tryptophan auxotroph *E. coli* B/r WP-2 try-. Increases in mutation frequencies were observed at dose levels from 5 μg/plate to a maximum dose of 100 μg/plate. Uchiyama's group also determined the DNA damaging potential of N-nitrosocarbaryl using the *Bacillus subtilis* rec-assay method. The endpoint of this assay is the difference in toxicity of two treated cell populations, *B. subtilis* Marburg 17, a recombination capable strain, and *B. subtilis* Marburg 45T, a recombination deficient strain. DNA damage was observed with N-nitrosocarbaryl at dose levels of 5 μg/plate and higher.

Egert and Greim (1976) found that N-nitrosocarbaryl was only slightly mutagenic to *E. coli* K12 and *S. typhimurium* TA-1538. However, significantly increased mutation frequencies were seen after metabolic activation with mouse liver microsomes. This result is curious since N-nitrosocarbaryl should be direct acting and several other workers report diminished activity when S-9 is added.

Marshall *et al.* (1976) examined the mutagenic activity of N-nitrosocarbaryl in the Ames assay using bacterial tester strains TA-1535, TA-1536, TA-1537 and TA-1538. N-nitrosocarbaryl was found to be a potent base-pair substitution mutagen causing a statistically significant increase in reversion with strain TA-1535 at the 0.5 μg/plate dose level. Positive results with strains TA-1537 and TA-1538 indicated that N-nitrosocarbaryl may be slightly active as a frameshift mutagen at higher concentrations. Presence of the

"S-9" liver homogenate decreased the mutagenic activity indicating that metabolic transformation results in inactive or lesser active metabolites or competed as a reaction target.

Regan *et al.* (1976) demonstrated that N-nitrosocarbaryl was able to induce DNA damage in cultured human cells as measured by unscheduled DNA synthesis by using ^{14}C methyl labeled and ^{3}H ring labeled N-nitrosocarbaryl. ^{14}C label was detected as associated with cellular DNA, whereas the ^{3}H label was not. N-nitrosocarbaryl has been repeatedly observed to be more active causing reversion of base-pair substitution sensitive strains TA-100, TA-1535. This evidence suggests that base-pa substitution type mutations are produced when the N-nitrosocarbaryl molecule is split and the resultant methyl group alkylates the DNA.

Blevins *et al.* (1977) found that the base-pair substitution sensitive *S. typhimurium* strains TA-100 and TA-1535 were reverted by N-nitrosocarbaryl without metabolic activation. The reversion frequency for TA-100 was increased by approximately 1.6-fold at 1.15 µg/plate and 6-fold at 11.5 µg/plate and for TA-1535 by about 3-fold at 1.15 µg/plate and 76-fold at 11.5 µg/plate. N-nitrosocarbaryl was not as active with the frameshift sensitive strains TA-98, TA-1537 and TA-1538. Maximum tolerated doses of carbaryl were given orally together with a 3 to 5 fold excess of sodium nitrite to screen rapidly for formation and mutagenicity of the N-nitrosocarbaryl in the intact mammalian organism (Seiler, 1977). Oral administration of carbaryl with sodium nitrite did not produce an elevated micronuclei count in the bone marrow of mice. Several explanations can be offered for these negative results. In the first place the pH in the mouse stomach may not be low enough to catalyze sufficiently the nitrosation of carbaryl (pH 4 - 5). N-nitrosocarbaryl might not have been formed in sufficient quantity to evoke a genotoxic effect in the bone marrow, however, the intraperitoneal injection of N-nitrosocarbaryl also led to negative results. The most likely explanation is that the highly labile N-nitrosocarbaryl is not biotransported to the bone marrow.

Ishidate and Odashima (1977) reported several different types of chromosome aberrations (80% aberrant cells) in Chinese hamster cells 24 hours after exposure to N-nitrosocarbaryl (0.015 mg/ml). The toxicity of N-nitrosocarbaryl was not reported.

Rickard (1980) studied the mutagenicity of N-nitrosocarbaryl. *S. typhimurium* TA-1535 and TA-100 produced direct activity but TA-98 did not. Addition of S-9 blocked the mutagenic activity. The authors demonstrated *in vivo* formation in rats and guinea pig. Dose response curves indicated N-nitrosocarbaryl to be the most active of the compounds studied by the author.

Eto *et al.* (1982) studied the mutagenicity of some possible metabolites of carbaryl including the dimethyl, hydroxymethyl, N-hydroxy and N-nitroso derivative. *S. typhimurium* and *B. subtilis* (rec-assay) detected activity only with N-nitrosocarbaryl. The activity was diminished by rat liver microsomal fraction (S-9).

Conclusions

- Carbaryl has been tested in a number of bacterial assay systems which measure genetic alterations in the form of specific locus gene mutations and produced no positive results.
- Carbaryl produced equivocal results at toxic levels in one report in cell culture. These results were not repeated by other workers.
- One study reported carbaryl produced an increase in DNA repair in cell culture at toxic doses, however, several others report negative results.
- Chromosomal damage has been reported for carbaryl *in vivo* in *Drosophilia* , at lethal doses, and in plants; and *in vitro* in human, rat and hamster cells at doses producing cell death.

- Mammalian *in vivo* studies in mice and rats with doses as high as 1,000 mg/kg in rats have been negative.
- No evidence exists to indicate carbaryl exposure increases the percentage of abnormal sperm or decreases sperm count in humans.
- N-nitrosocarbaryl is mutagenic in several test systems.
- Metabolic activation has variously been reported as increasing, decreasing or not changing mutagenic activity of N-nitrosocarbaryl. N-nitrosoamides as opposed to N-nitrosoamines should not require activation.
- N-nitrosocarbaryl does not biotransport to produce elevated micronuclei in the bone marrow of mice.
- Carbaryl is not a mutagenic hazard to man.

IV: ONCOGENICITY

Summary

Oncogenicity is a term used to define the ability of an agent or xenobiotic to induce benign and malignant neoplasms. A number of long term oncogenicity feeding studies testing carbaryl have been conducted. These studies have utilized rats, mice and dogs as test animals. No oncogenicity has been attributed to carbaryl in any of these studies. Many of the study protocols were not optimal when compared to current study standards, but the total data base provides more than sufficient evidence conclude that carbaryl lacks carcinogenic activity.

Carbaryl has been demonstrated to undergo an acid catalyzed reaction with nitrite ion to form N-nitrosocarbaryl. N-nitrosocarbaryl has been demonstrated to be moderately potent contact carcinogen. N-nitrosocarbaryl is not formed, at all or at least not formed in carcinogenic quantities, at the maximum doses of carbaryl or carbaryl plus nitrite permitting long term survival in *in vivo* feeding bioassays (see VI: Human Exposure).

Carbaryl

Mice. Carpenter *et al.*, (1957) administered 20 weekly subcutaneous injections of 0.20 ml of 0.05% carbaryl suspended in 0.25% agar to 30 C3H mice in an attempt to induce lung tumors. One control group received only the agar injection and the second control group received no injections. The mice were sacrificed at 8 months of age. Excess lung tumors were not found.

Weil and Carpenter (1962) reported that no tumors were seen in a 24-month skin painting study in mice with carbaryl administered as the wettable powder formulation, SEVIN® 85-S. The diluted concentration was 48% in water.

Weil and Carpenter (1962) fed diets containing 0.0, 10 and 40 ppm carbaryl to groups of 48 male and 48 female CD mice. The mice were housed four to a cage and observed daily. A high incidence of mortality was experienced in all groups, especially during the third half-year of dosing. In order to provide a reasonable sample for histological evaluation, 12 mice of each sex and each dosage group were killed after 80 weeks. The incidence of organ involvement and tumor type in the unautolyzed mice that died and in those sacrificed after 80 weeks was similar in all groups. Weil and Carpenter concluded that the consumption of 10 or 40 ppm carbaryl in the diet of CD mice for 80 weeks was not associated with increased tumor production.

Shimpkin *et al.* (1969) explored the carcinogenic activity of carbaryl using the pulmonary tumor-induction method and Strain A mice for bioassay. Carbaryl induced lung

tumors in 40% of the mice with 0.7 lung tumors per mouse. The results were not statistically significant and considered to be equivocal by the authors.

Innes *et al.* (1966) tested the tumorigenicity of carbaryl by continuous oral administration or single subcutaneous injection to both sexes of two hybrid strains of mice. Carbaryl was administered as a single subcutaneous injection (100 mg/kg in DMSO) or orally (4.64 mg/kg in gelatin) via stomach tube from the 7th to the 28th day of age; and thereafter mixed in the feed. Maximum tolerated doses were utilized. A variety of tumors (lymphomas, pulmonary, liver) were seen in small numbers of mice. The study was negative with respect to carcinogenesis for carbaryl.

A.J. Triolo (1978) reported in a U.S. EPA document (Anderson,1980) a study which attempted to evaluate the promoting or co-carcinogenic ability of carbaryl to enhance the incidence of forestomach tumors in Ha/ICR female mice and lung tumors in A/J female mice treated with the carcinogen benzo[a]pyrene. This experiment is very important when evaluating the possible *in vivo* nitrosation of carbaryl since both N-nitrosocarbaryl and benzo[a]pyrene produce stomach tumors. If oncogenic levels of N-nitrosocarbaryl was produced, an enhanced effect should have been observed. Dietary levels of carbaryl up to 2,000 ppm did not increase the incidence of forestomach tumors in mice treated with 300 ppm benzo[a]pyrene in the diet for 12 weeks. A/J mice were also given 3 mg benzo[a]pyrene *per os* on days 7 to 21 and 1,000 ppm carbaryl in the diet for 20 additional weeks. A suggestive, but not statistically significant, increase in lung tumor incidence was produced when the carbaryl plus benzo[a]pyrene treated group was compared to the benzo[a]pyrene control. The inconclusive experiment was repeated and the incidence at 16 weeks in the carbaryl plus benzo[a]pyrene group (16/34) was similar to that in the benzo[a]pyrene control group (16/31). Neither an oncogenic nor co-carcinogenic effect was not demonstrated.

Rats. Carpenter *et al.* (1961) studied the effect of carbaryl on metabolism, cholinesterase inhibition, alleviation and aggravation of symptoms with atropine sulfate and by pyridine-2-aldoxime methiodide (2-PAM). Neuromuscular degeneration, carcinogenic activity, and sensitizing propensity were not observed. The responses of several species to single doses by oral, parenteral, percutaneous, and respiratory routes were studied. A 2-year feeding study demonstrated that 200 ppm in the diet was tolerated by the rat with no significant deviations from controls.

Lijinsky and Taylor (1977) treated pregnant female rats by gavage with a total of 300 mg of carbaryl during a 10 day period. No malignant tumors were induced in the females, nor in their offspring, during their natural lifespan. Pregnant, and non-pregnant female rats were given a suspension of carbaryl and sodium nitrite solution on several successive days until a total of 90 mg carbaryl and 120 mg sodium nitrite was administered to each adult rat. Neither the female rats, nor their offspring, developed malignant tumors as a result of the treatment with the mixture of carbaryl and sodium nitrite.

Dogs. Weil and Palm (1958) fed groups of 3 or 4 dogs, consisting of Cocker or Basenji hybrids or crosses, capsules containing 0.0, 0.45, 1.8 and 7.2 mg/kg of carbaryl 5 days per week for one year. These dosages were approximately equal to 0, 25, 100 and 400 ppm of carbaryl in the dry diet. Weight gain, mortality, liver and kidney weight at sacrifice and micropathology of sections of many organs were examined. Various hematological and biochemical determinations were made at five intervals, while red blood cells and plasma cholinesterase were measured at 20 intervals.

Carbaryl dosed dogs did not differ significantly from their controls in any of the parameters measured. Carpenter (1961) studied carbaryl by the same protocol described above for rats. Dogs tolerated 400 ppm in their diet with no differences detected when compared to control animals.

Russian Literature. None of the carcinogenicity studies referenced in the United Nation Environmental Program (UNEP) Review of the Russian Literature on carbaryl

(1982) meet contemporary standards for conducting chronic studies, however they are included in this review to provide convenient reference.

Makovskaya *et al.* (1965) treated 400 mice with weekly intraperitoneal injections of carbaryl for up to 2 years. They concluded that carbaryl did not induce neoplasms.

Zabezhinski (1970) studied the carcinogenic effect of injected and orally administered α and β carbaryl in rats for 33 months and to mice for 24 months. The number of animals utilized was small and a number of the animals died early due to other diseases. They concluded that β-carbaryl had very slight or no carcinogenic effect in mice but that it induced sarcomas at the site of injection in rats. The authors also concluded that α-carbaryl was not carcinogenic.

A single Russian article (Andrianova and Alekseev, 1970) reported that carbaryl induced tumors in rats. Carbaryl was administered in the diet and by injection. This study was compromised beyond the point of serious consideration. Many essential details including; a description of pathology on 80% of the total animals (which died prior to 22 months); compound purity; and the identity of impurities and isomeric content were not described.

N-Nitrosocarbaryl

Rats. Eisenbrand *et al.*, (1975) administered a single subcutaneous (sc) injection of 1,000 mg/kg of N-nitrosocarbaryl suspension to 8 male and 8 female Wistar rats. Of the 16 rats given the single sc dose, 14 died by day 450 with sarcomas at the site of injection. Thirty-seven Wistar rats of both sexes were given single oral doses of 200-1,500 mg/kg. In the animals administered the compound orally, no tumors were evident in 21 months. Any effect produced by N-nitrosocarbaryl apparently was not persistent enough, when administration was a single oral dose, to produce a positive response. This experiment reinforces the concept that N-nitrosocarbaryl is active at the site of first resident contact requires repeated administration and does not persist long enough to be transported to other tissue when injected or injested (Magee 1969).

Linjinsky and Taylor (1976) administered N-nitrosocarbaryl, by gavage in olive oil solution, to groups of 12 female Sprague-Dawley rats. The dosage was 0.2 ml of 0.11m solution once a week for 10 weeks resulted in a total dose of 0.22 millimoles (40mg). The rats given N-nitrosocarbaryl developed a high incidence of tumors (75%). The tumors induced were almost all invasive squamous carcinomas of the stomach. When a six fold larger total dose of N-nitrosocarbaryl, 1.3 millimoles (260 mg), was given to male rats twice weekly for 20 weeks, it produced an equal but not higher incidence of stomach tumors than the lower dose (40 mg) had produced in the females. The male animals with tumors died earlier. This study suggests the vehicle may be important in providing tissue penetration, prolonging bioavailability or both. It is not clear whether differences observed in males and females were due to sex sensitivity or dosage frequency.

Preussman *et al.* (1976) administered 130 mg/kg of N-nitrosocarbaryl to "equal numbers" of 100-day-old male and 200 g female Sprague-Dawley rats twice weekly by gavage until death. Twenty male and female control animals were available for comparison. Animals were subjected to both gross and histopathologic examinations. Hyperkeratoses, papillomas, and squamous cell carcinomas were found in the forestomach in 17 of 32 treatment rats, but none were present in control animals. The mean tumor induction time was 167 days. This study will be used to estimate risk in VII: N-Nitrosocarbaryl Cancer Risk Analysis.

The Technical Panel on Carcinogenesis (Mrak,1969) examined the available reports on tests of tumorigenicity conducted on carbaryl and concluded that no action needed to be taken to alter current practices. Carbaryl was judged "not positive" for tumor induction on the basis of long term tests conducted under adequate conditions in two or more species.

Conclusions

Although no single test reviewed is ideal, this reviewer suggests that the numerous studies conducted provide more than adequate data to conclude that carbaryl does not induce cancer in animals.

N-nitrosocarbaryl is not a potent carcinogen. Subcutaneous injection of N-nitrosocarbaryl can cause sarcomas at the site of injection. Single oral doses of 1,500 mg/kg are not carcinogenic. A 40 mg/kg dose by weekly regime to female rats yields similar results. A 130 mg/kg and 260mg/kg dose by bi-weekly regime to male rats is highly carcinogenic. Olive oil as a carrier may protect against decomposition and promote absorption by stomach tissue.

If 0.1% of carbaryl is the maximum N-nitrosocarbaryl produced *in vivo* (See Rickard and Dorough, 1984 in VI: Human Exposure) animals would have to consume several thousand multiples of lethal doses of carbaryl and nitrite at each individual exposure in order to obtain quantities of reactants necessary to produce carcinogenic doses of N-nitrosocarbaryl via the process of *in vivo* nitrosation. Single oral doses of N-nitrosocarbaryl, equivalent to that predicted to be formed by *in vivo* nitrosation of 10,000 LD_{50}'s of carbaryl by 1,000,000 LD_{50}'s of nitrite, produced no effect. Any postulated carcinogenic risk due to exposure to N-nitrosocarbaryl theoretically formed *in vivo* by nitrosation of ingested carbaryl by nitrite, is intuitively vanishingly small or indeed non existent. (See VII: N-Nitrosocarbaryl Cancer Risk Analysis).

V: IMMUNOTOXICOLOGY

Summary

The administration of carbaryl, or any xenobiotic, at doses resulting in overt poisoning can be expected to result in a variety of effects on the immune system. Carbaryl when administered at doses not causing overt clinical signs has been reported to produce a variety of reversible and non-life threatening effects to the immune system. Several authors have suggested the observed effects were due to subtle treatment-related stress. Lifetime exposure to carbaryl has not resulted in increased disease in rats and mice. Carbaryl does not serve as a promoter of stomach cancers induced by benzo[a]pyrene. Most studies in rabbits, mice and rats, at doses permitting survival, have not produced significant effects on the immune system. No positive effects have been observed in man. Carbaryl does not appear to represent an immunological risk factor.

Viral enhancement is defined as the induction of an increase in virus production or replication. A number of researchers have demonstrated viral enhancement in tissue culture by prior incubation with xenobiotics. Carbaryl can enhance *Herpes varicella-zoster* the virus associated with chicken pox and shingles. Carbaryl did not enhance *Herpes simplex* or murine influenza virus. Carbaryl and α-naphthol compromise interferon synthesis in goldfish permitting viral enhancement. Long term *in vivo* feeding studies have not enhanced viral infections. Carbaryl does not enhance transformation of BALB/5T3 fibroblasts in culture or the expression of indogenous murine leukema virus. The appropriateness of extrapolating from enhancing effects observed in cell culture systems to predictions of similar effects in humans has not been established. Epidemiologic studies in geographical areas where carbaryl has been used extensively have not detected an increase in Reye's syndrome.

Introduction

The immune system is a complex interdependent and cooperating set of subsystems which provide the body's primary defense against infectious diseases and cancer. The various components of the immune system are individually susceptible to enhancement as well as impairment by foreign materials. A wide range of xenobiotics have been reported to produce direct and indirect modifications of immune homeostasis. Factors contributing to homeostatic pertubation range from non specific stress to direct tissue toxicity. Any event or agent producing a life threatening condition can be expected to, and usually will, influence the immune system. The goal of immunotoxicology is to detect the type, magnitude and duration of effects on the immune system and to determine if such effects impair the organism's intrinsic capability to combat cancer and infectious disease.

In Vivo Studies

Dinoyeva (1956) studied the dynamics of the changes in immunological structures of lymphatic follicles in the spleen during carbaryl administration. Changes in the immunological structures of the spleen were studied in 30 to 35 day old albino rats treated daily with 1.5mg/kg of carbaryl for 6 months. A positive control group received daily 2 mg/kg doses of cyclophosphamide. Plethora of the spleen and retardation of the development of the lymphatic follicles were observed in the groups treated with carbaryl and cyclophosphamide. The carbaryl-treated group showed few changes in the structure and weight of the thymus in contrast to the positive effects on the cyclophosphamide-treated group.

Moreinis and Estrin (1965) coadministered an LD_{15} of murine influenza virus strain A-PR_8 and 500 mg/kg carbaryl. The animals were studied histologically. The combined effect was equal to the sum of the response to the individual virus and carbaryl. No enhancement was observed.

Perelygin *et al.* (1972) studied the effects of carbaryl on immunological response in rabbits and albino rats. Carbaryl at 0.1 mg/kg failed to produce any stable changes in immunological reactivity. Carbaryl at 20 mg/kg produced a decrease in phagocytic activity of leukocytes, antibody formation and protective properties of the serum. A transient increase in activity was followed later by a decrease in response. Carbaryl at 200 mg/kg caused a prolonged and gradual decrease in reactivity.

Street and Sharma (1974) fed carbaryl for a 28-day preantigen pretreatment period. Antigens were administered on day 29 into the foot pad (sheep erythrocytes plus Freund's complete adjuvant) and the status of the immune system was evaluated over the ensuing 28 days while treatment was continued. Total dosages of carbaryl up to 8.38 mg/kg, yielded no consistent indications of immune suppression.

Street and Sharma (1975) repeated their earlier experiment with rabbits at 0.3, 1.08, 2.30 and 8.38 ppm of carbaryl. Treatment resulted in a reduction of germinal centers in the spleen, and atrophy of the thymus cortex. Results were inconsistent at low doses. Anticipated challenge related antigen-induced increase in serum globulin was consistently decreased by carbaryl treatment but not dose related. Significant changes due to carbaryl treatment were observed only at 10 days post antigen. No effects were detected in plasma cell count in popiteallymph nodes, hemolysin and hemaglutinin titers, skin sensitivity to tuberculin, leucocyte count, growth, food consumption, or body weight to organ weight ratios. The authors suggested that the immunosuppressive effects observed might be due to physiological stress but noted that such stress was not mediated via the adrenals.

Olefir and Minster (1977) studied the natural immunity of white rats treated with 1/20-1/1000 LD_{50} doses (40 to 0.8 mg/kg) of carbaryl daily for 4.5 months. An inverse

relationship was found between level of exposure and serum complement-fixing activity and lysozyme level, as well as the immunological function of the reticuloendothelial system, neutrophils, skin and mucosa. Most nonspecific immunological parameters showed insignificant changes. A specific loading test (oral administration of 1/5 LD_{50}) and a nonspecific loading test (administration of pyrosenal) at the end of the 4.5 month treatment period showed no significant differences between the controls and treated groups.

Disorders in immune response were reported in rats after a 3.5 month treatment with daily 1/20 LD_{50} doses of carbaryl (Olefir, 1978). Carbaryl did not affect immunogenesis when administered at daily 1/1000 LD_{50} doses. Sufficient data were not available to provide a definition of disorders.

Roszkowski (1978) conducted immuno-morphological investigations on the effect of carbaryl on immune reactions of rabbits. Carbaryl, when administered to rabbits in subtoxic doses (actual dose not available) for 90 days, had no depressive effect on immune reactions as expressed by hemagglutinin and hemolysin levels, the number of plaque forming cells, and spleen lymphatic tissue reaction. An increase of hemolysin serum titer and spleen lymphatic tissue reaction was reported for all treated rabbits.

Akhundov *et al.* (1981) studied the effect of carbaryl on immunologic reactivity of rabbits and guinea pigs given oral doses of 15 mg/kg carbaryl for 42 days. The autosensitizing effect of heated vaccine from *Salmonella typhimurium* was tested. The vaccine at doses of 250 or 500 million microbial cells was administered 3 times at 5 day intervals, beginning on day 21 of the experiment. Carbaryl enhanced the autosensitizing effect of the vaccine. Experiments in which guinea pigs were treated with 15 mg carbaryl/kg daily for 3 months were interpreted as producing macrophase migration and inhibition of delayed hypersensitivity.

Pipy *et al.* (1983) evaluated the cellular and humoral mechanisms of carbaryl-induced reticuloendothelial phagocytic depression in rats. The simultaneous injection of carbaryl (3.8 to 30 mg/kg body weight) and colloidal carbon results in competitive phagocytization by the reticuloendothelial cells in favor of the carbon particles. Colloidal carbon produced a slight and variable increase in carbaryl (either opsonized or non-opsonized) uptake by the spleen and lungs. The authors concluded on the basis of "enzymatic investigations" that the reticuloendothelial systems depressed ability to incorporate colloids during carbaryl treatment could be due to a defect in the activity of macrophage membrane-bound serine esterase.

The effect of carbaryl on an experimental *Erysipelothrix rhusiopathise* infection in rats was studied by Shabanov *et al.* (1983). An iv inoculation with 1.5×10^8 *E. rhusiopathiae* cells/rat was preceded by 30 daily oral administrations of carbaryl in doses increasing gradually from 2 to 5 mg/rat. Septicemia and mortality increased from 14 to 36%. Survival time decreased by 50%. The administration of carbaryl hastened the onset of bacteremia from the 10th-12th to the 3rd-4th day after infection and aggravated histologically observed effects of septicemia.

Lox (1984) studied in the rat the effects of 30 days of exposure to 10 ppm carbaryl in the drinking water on clotting factors. Thirty male Sprague-Dawley rats weighing between 250 and 300 g were divided into two groups of 15 rats and placed on an *ad libitum* solution of tap water or 10 ppm of carbaryl in tap water. The rats were treated for 30 days and then sacrificed. Rats consuming the 10 ppm *ad libitum* carbaryl drinking solution decreased their water consumption without effect on body weight gain. Slight but significant decreases in platelet count and factor VII clotting activity were reported. Microscopic evaluation of the liver tissue were reported to reveal a number of treatment related changes including hepatocyte degeneration, central vein congestion, some leukocytic infiltration, and vacuolization of the cytoplasm. The results of this study are significant in that levels of carbaryl which were capable of producing frank liver toxicity produced minimal effect on clotting factors.

Wiltrout *et al.* (1978) conducted studies on the effects of dose and time of exposure relative to immunization and subsequent immune response in female BALB/c mice following administration of 0.5 mg oral doses of carbaryl. Carbaryl was administered five days before, on the day of, and two days after immunization and fed as daily doses for eight and 28 days before immunization. Carbaryl induced significant suppression of the humoral immune response only at near-lethal doses.

Zeakes *et al.* (1981) studied the increased susceptibility of quail to infection by *Histomomas meleagridis* after exposure to carbaryl. Quail given heterakid eggs but no carbaryl became infected with cecal histomonads, but there was no pathological histomoniasis. Quail given 10 μg of carbaryl for five days behaved normally but at necropsy had slightly discolored livers. Quail were given various doses of heterakid eggs and carbaryl increasing from 2.5 to 50 μg. Quail given various doses of heterakid eggs and 10 μg/day of carbaryl developed pathological histomoniasis and mortality rates of 36 and 63%, respectively.

Roszkowski *et al.*, (1976) studied the effect of carbaryl on immune response in chickens treated orally with 500 mg carbaryl/kg 1 day before, on the day of, or 1, 2, or 3 days after immunization. Tests with 1% sheep red blood cells showed inhibited hemolysin production and decreased the numbers of splenic germinal centers. Hemagglutinin levels were not altered by the treatment.

Roszkowski (1979) conducted immunomorphological investigations on the effect of carbaryl on chickens. Carbaryl when administered to chickens in subtoxic doses (actual doses not available) for 90 days had no depressive effect on immune reactions represented by hemagglutinin and hemolysin titers, the number of splenic plaque-forming cells or splenic lymphatic tissue reaction.

Animals used in long term bioassays of carbaryl are carriers of many viruses. If carbaryl were an *in vivo* enhancer one would expect viral disease to have been expressed to a greater extent in the treated than in the control. Survival data and comments of researchers in specific reports lead this author to the conclusion that viral enhancement by carbaryl has not been demonstrated *in vivo* test systems.

In Vitro Studies

Quarles and Tennant (1975) reported that N-nitrosocarbaryl, but not carbaryl, transformed BALB/3T3 fibroblasts. Neither chemical induced the complete expression of endogenous murine leukemia virus. Transformed cells by loss of contact inhibition, change in morphology, growth in soft agar, growth to higher saturation densities, and tumorigenicity in normal newborn and irradiated weanling mice and athymic (nude) mice. Transformed clones were found to be negative for expression of RNA tumor virus antigens, viral reverse transcriptase, and infectious virus. Thus, it appears that N-nitrosocarbaryl can transform BALB/3T3 cells to tumorigenic cells with altered biological properties, however the RNA tumor viruses in the transformed cells are not completely activated. Expression of viral antigen in the transformed cells was inducible by iododeoxyuridine, indicating that the endogenous viral genome was retained in an unexpressed state.

The replication of goldfish virus-2 (GFV-2) in CAR cells was reported as enhanced *in vitro* by pretreatment of piscine cultures with subcytotoxic concentrations of carbaryl (Shea, 1983). This phenomenon was dependent on time and temperature, did not involve differential adsorption of virions, and did not result from direct interaction of pesticide and virion. It was suggested that metabolic interaciton between CAR cells and carbaryl was responsible for enhancement .

Jerkofsky and Abrahamsen (1983) reported that the ability of various human Herpes viruses to be enhanced by the pretreatment of human embryonic lung cells with carbaryl differed according to the virus tested. Different strains of *Varicella-zoster* virus produced

different patterns of susceptibility to enhancement. Laboratory-adapted strains were less sensitive to enhancement than were wild-type strains recently isolated from clinical specimens. The related human *Herpes simplex* viruses types 1 and 2 and cytomegalovirus were negative for susceptibility to enhancement when either laboratory-adapted or wild-type strains were tested. No difference in the pattern of susceptibility was detected when virus yields were determined by cell-associated or cell-free virus assay or when the input multiplicity was varied 10-fold.

Abrahamsen and Jerkofsky (1983) also reported on the enhancement of *Varicella-zoster* virus by carbaryl. The viral enhancement assay consisted of monolayers of HEL cells exposed to carbaryl for 20-24 hours at 37°C. It was necessary to expose cells to the enhancing chemical during the period of virus replication to detect enhancement. The monolayers were then washed to remove the carbaryl. Virus-infected cells were added and the cultures were incubated in fresh growth medium in the absence of carbaryl until viral cytopathic effects were obvious. Cultures were then harvested and assayed for virus yields. The optimum time for the pretreatment was 20 to 24 hours. Maximum enhancement of virus expression occurred 48 to 72 hours postinoculation. I-Naphthol, a metabolite of carbaryl, was also capable of enhancing virus replication, but the treated cells could not pass on to daughter cells the ability to produce increased amounts of virus.

In an effort to determine the mechanism for enhancement of GFV-2 infected CAR cultures Shea and Berry (1984) tested for interferon synthesis and for a reduction in interferon synthesis in infected cultures which had been pretreated with carbaryl. Interferon production can be produced by the goldfish-derived CAR cell line in response to infection by goldfish virus-2. Supernatants of infected cultures provided antiviral protection to CAR cells and another cell line derived from goldfish, ABIII. The protective factor retained activity after ultracentrifugation, dialysis, freezing and thawing, acid treatment (pH 2), or heating to 56°C but was sensitive to trypsin. Supernatants of infected cultures did not affect adsorption of virus. Carbaryl-treated cultures were found to synthesize reduced levels of interferon. Supernatants from infected cultures, not treated with carbaryl, were found to provide 10 times as much antiviral protection as supernatants form uninfected cultures with or without carbaryl pretreatment. In contrast, carbaryl-treated cultures provided only twice as much antiviral protection as uninfected cultures.

A similar relationship was observed when assaying the amount of infectious viral progeny synthesized in the presence of the various supernatants. Virtually identical levels of virus were synthesized with either stock medium or supernatants from uninfected cultures, both with and without carbaryl pretreatment. Only 10% of the amount synthesized in stock medium was synthesized in the presence of the supernatants from infected cultures not pretreated with carbaryl. Far less antiviral protection was provided by the supernatants of infected cultures which were pretreated with carbaryl as these secondary CAR cultures synthesized over 60% of the amount of virus synthesized in control cultures.

Shea and Berry (1984) proposed that 1 ppm carbaryl imposes a mild inhibition of (certain unknown aspects of) cellular metabolism, clearly undetectable by the parameters of cytotoxicity employed by this study, and that this mild suppression is rendered functionally acute with respect to interferon biosynthesis by subsequent viral infection.

Reye's Syndrome

Bradford and Parker (1971) stated that Reye's syndrome usually begins with a febrile upper respiratory tract infection followed by the onset of coma. Patients typically present with flexed elbows, extended legs, and a clenched fists posture. Common laboratory findings include a low blood sugar, low cerebrospinal fluid sugar, metabolic acidosis, elevated blood urea nitrogen, ketonuria, aminoaciduria, and marked elevations in SGOT and blood ammonia. Children who develop this illness under the age of two years often die.

The government of New Brunswick, Canada was concerned about the significance of the medical research involving viral enhancement. They questioned if there was a relation between forest spraying for the control of spruce budworm and the incidence of Reye's syndrome in children. The Minister of Health in New Brunswick appointed a Panel of six scientists from outside New Brunswick to examine the relevant literature and expert opinions. The Panel concluded that while there may be cause for concern about the possibility that exposure to certain commonly used chemical products may potentiate viral infections, they could not find evidence linking the occurrence of Reye's syndrome in New Brunswick to the forest spraying program. Exposures to many common chemicals would be expected to be of more general significance than carbaryl exposures resulting from forest sprays. The Panel stated that more comprehensive studies were needed to completely allay their concerns about viral enhancement by xenobiotics and made recommendations for additional research (Schneider *et al.*, 1976).

Conclusions

The administration of carbaryl or any xenobiotic at doses resulting in overt poisoning can be expected to result in a variety of effects on the immune system. Carbaryl when administered at doses not causing overt clinical signs has been reported to produce a variety of reversible and non-life threatening effects to the immune system. Several authors have suggested the observed effects were due to subtle treatment related stress. Most studies in rabbits, mice and rats, at doses permitting survival, have not produced significant effects on the immune system. No positive effects have been observed in man. Carbaryl does not appear to represent an immunological risk factor.

Carbaryl produces interesting effects in several *in vitro* systems. Toxic actions of carbaryl *in vivo* can disrupt the immune systems ability to combat certain viral infections. There is no evidence that low doses of carbaryl are likely to produce viral enhancement in humans. No epidemiological evidence links carbaryl and Reye's syndrome.

VI: HUMAN EXPOSURE

Summary

Respiratory and dermal exposure has been studied in individuals involved in the manufacture, formulation, packaging, distribution and application of carbaryl. FDA Market Basket Surveys provide information on the extent to which food may serve as a source of human exposure. Additional studies have provided data on the toxic effects of carbaryl following voluntary oral ingestion and revealed details of human metabolic pathways. Reports on incidents of intoxication from carbaryl, over approximately a 20 year period, indicate that the majority of the very few cases reported are due either to suicide attempts or accidents which resulted from the transfer of carbaryl to innappropriate containers attractive to children. Mortality is rare despite general availability, frequent and diverse use. Reviews covering these subjects are provided in reports by NIOSH (1976), EPA (1977), Harry (1977), EPA (1980), and Weston (1982).

Metabolism of Carbaryl in Man

Most of the metabolites formed by humans are not unique and have also been formed by tissue cultures, cell cultures, and enzyme preparations from rabbit, rat and mouse. Most human metabolites have also been isolated from the urine of treated chickens, rats and rabbits. *N*-hydroxycarbaryl and 1-hydroxy-5,6-dihydro-5,6-dihydroxy-naphthylene have been isolated exclusively from rodent preparations (Dorough and Casida, 1964; Leeling and Casida, 1966).

Chromatographic and fluorescence methodologies that successfully distinguished four naphthyl-containing compounds in rat urine detected only 1-naphthyl glucuronide (25 ppm) and 1-naphthyl sulfate (5 ppm) in the urine of men who packaged carbaryl (Knaak *et al.,* 1965).

Knaak *et al.* (1968), administered 2 mg/kg carbaryl to two men. Five urinary metabolites were identified including free and the glucuronide and sulfate conjugates of 1-naphthol, 4-hydroxycarbaryl and a minor amount of 1-naphthyl methylimido carbamate-0-glucuronide. Following a single dose of 2 mg/kg, 26 and 28% was recovered from urine within 4 days. The metabolites were: 4-(methylcarbamoyloxyl)-1-naphthyl glucuronide (about 4%), 1-naphthyl glucuronide (about 15%), and 1-naphthyl sulfate (about 8%). There was only qualitative evidence of 1-naphthyl-methylimidocarbonate-*O*-glucuronide. One or more unidentified neutral compounds also were present. A slightly higher proportion of the dose from the same samples (37.8%) was calculated as recovered when a colorimetric method sensitive to total 1-naphthol was utilized.

Human embryonic lung cells in culture metabolize carbaryl to form 1,4-naphthalenediol and N-glucuronides of 4-hydroxycarbaryl and of 5,6-dihydroxy-5,6-dihydrocarbaryl (Baron and Locke, 1970). Many of the metabolites formed by human cells have also been recovered from chicken urine in addition to 5,6-dihydrocarbaryl, and 1,5,6-trihydroxynaphthylene (Paulson *et al.,* 1970). Human tissues (Chin *et al.,* 1974) and rats (Sullivan *et al.,* 1972) form both naphthyl glucuronide and naphthyl sulfate. Rats at least, excrete a cysteine conjugate (Bend *et al.,* 1971). Most of the compounds formed by human embryonic lung cells have also been recovered from the medium used to incubate human liver slices (Sullivan *et al.,* 1972) or from the urine of volunteers.

When ^{14}C-carbaryl was applied to the forearms of volunteers the rate that radioactivity appeared in the urine progressively increased from 8 to 12 hours after application and then gradually declined. Activity remained greater in samples collected 96 to 120 hours after application than in those collected in the first 1 to 4 hours after application. Only 7.4% of an intravenous dose of ^{14}C-carbaryl was detected in the urine (Feldmann and Maibach, 1974).

Lin *et al.* (1975) using the same human embryonic lung cell line as Baron and Locke (1970), but extracting with ether, identified the same three metabolites and in addition, 1-naphthol, 5-hydroxycarbaryl, and 1,5-naphthylenediol. Several unknowns remained to be identified. Because β-glucuronidase did not free the aglycones from conjugation, it is likely that the conjugates were other than *O*-glucuronides. The incubation was carried out for 72 hours at a concentration of 1 ppm. Under these conditions, cell growth was not decreased, and 99% of the radioactivity was recovered, 70% as metabolites of all kinds including 30% that were water-soluble (Lin *et al.,* 1975).

Myers (1977), determined that 41.5 to 52.7% of an oral dose of carbaryl administered to 9 human volunteers was excreted in the urine by 49 hours. The author showed a good correlation between the dose administered orally and urinary metabolites of carbaryl but was uncertain of the application of the technique for monitoring dermal and respiratory absorption in the work place.

Studies conducted to date do not provide the information necessary for material balance or rate calculations. However, it is clear that carbaryl is rapidly detoxified and the resultant metabolites are excreted predominately in the urine.

Monitoring of Exposure

Best and Murray (1962) determined the concentration of carbaryl in air, 1-naphthol levels in urine and acetylcholinesterase levels in blood of workers in a pesticide manufacturing plant. During one period, 41% of the urine samples contained in excess of 1 mg of total 1-naphthol per 100 ml of urine. Air concentrations of carbaryl ranged from 0.23 mg to 31 mg per cubic meter of air. Depressed cholinesterase levels were

occasionally detected but signs or symptoms of anticholinesterase activity were absent. Best and Murray (1962) were not able to correlate air concentrations of carbaryl with excretion of 1-naphthol because of the limited number of air samples and apparent variations in urinary excretion. The most heavily exposed group of workers yielded an average urinary concentration of 1-naphthol of 18.5 ppm. The sporatic depression of cholinesterase activity was not associated with systemic poisoning. A urine concentration of 18.5 ppm would suggest an absorbed dosage of about 40 mg/man/day or about 0.55 mg/kg/day. Exposure continued during the entire work day permitting detoxification to occur simultaneously with absorption. The exposure value of 0.55 mg/kg/day is less than 0.7 mg/kg/day calculated from the TLV of 5 mg/m^3. (NIOSH, 1976)

Jegier (1964) determined respiratory and dermal exposure to carbaryl during spraying of orchards, small fruits, vegetables and grain. Average respiratory exposure was determined to be 0.29 mg/hr and the average dermal exposure was calculated to be 25.3 mg/hr. The maximum total exposure (dermal plus respiratory) was reported as 31 mg/hr. About half of the operators involved in all spray operations (48%) failed to use protection.

Dermal and respiratory exposure was measured in a small group of spray operators in a large fruit growing region of New South Wales, Australia (Simpson, 1965). Respiratory exposure was reported as 0.5 mg/hr and was considered to indicate little hazard.

Eighty-five percent water-wettable carbaryl powder was applied as a 5% spray for six hours by eight spraymen and two supervisors. Application was to the inside of houses in a small Nigerian village where carbaryl was used regularly. The rate of surface application was calculated to be 2 g/m^2 which is equal to an agricultural field use of 20 lbs/acre. The only untoward physical symptom was a pronounced skin rash which developed on a sprayman whose back was directly splashed with carbaryl. A 15% depression of plasma cholinesterase was the average found in the spraymen on day one. Plasma cholinesterase values for 64 of the 95 villagers showed an average decrease of 8%. The spraymen showed no detectable increase in urinary excretion of 1-naphthol. Villagers sampled 1 week after spraying demonstrated a significant increase in urinary 1-napthol from a baseline of 30.5 ppm to 50.3 ppm (Vandekar, 1965).

A single dose of 0.5, 1.0, and 2.0 mg/kg was ingested by two men at each level. Neither subjective nor objective effects were noted (Wills *et al.,* 1968). Five men received 0.06 mg/kg/day carbaryl and six additional men received between 0.12 to 0.13 mg/kg/day for six weeks. No abnormalities in BSP, EEG, plasma and erythrocyte cholinesterase, complete blood count, blood, or urine chemistry were attributable to carbaryl at the lower dosage. An increase in the ratio of amino acid nitrogen to creatinine in the urine at the high dose was detected and interpreted as an indication of a slight and reversible decrease in the ability of the proximal convoluted tubules to reabsorb amino acids (Wills *et al.,* 1968). Wills *et al.* (1968) also studied the effects of daily p.o. doses of carbaryl (0.06 or 0.12 mg/kg) in the male human volunteers. After 6 weeks of exposure, no EEG changes were found which were attributable to carbaryl exposure.

Workers in three plants formulating 4% and 5% carbaryl dust yielded mean values of 73.9 mg/hr for dermal exposure and 1.1 mg/hr for respiratory exposure. Spraymen operating tractor-drawn power air-blast equipment during application of 0.045% to 0.06% carbaryl spray to fruit orchards yielded values of 59 mg/hr for dermal exposure and 0.09 mg/hr for respiratory exposure (Comer *et al.,* 1975). The concentration of 1-naphthol in the urine of formulators and applicators varied from 0.2 to 65 ppm with a mean of 9 ppm. The rate of excretion varied from 0.004 to 3.4 mg/hour, with a mean of 0.5 mg/hour, equivalent to 0.7 mg of carbaryl per hour or 6 mg/man/8-hour day. The concentration increased gradually during the work day, reached maximal level in the late aftermoon and early evening, and returned to lower levels before the start of the next day's work. Comer *et al.* (1975) calculated the highest exposure level and determined that it represented only 0.4% of a toxic dose per hour of work. Comer *et al.* (1975) concluded that dermal absorption of carbaryl is likely very limited since urinary excretion accounted for less than 1% (6 mg per 8 hour day) of the total dermal exposure (600 mg per 8 hour day).

Shpirt (1976) studied the dynamics of the immunological shifts in workers exposed to pesticides. Changes in the specific immunity were studied in 674 sugar beet growers having short-term exposure to pesticides. Antibodies to DDT, Zineb, and BHC were found. Antibodies to carbaryl could not be identified positively in the exposed workers.

Whorton *et al.* (1979) examined semen samples from 47 carbaryl production workers with at least one year of experience. A group of chemical plant workers, not previously exposed to carbaryl served as an external control. The authors found no association between sperm count levels and either the degree or duration of exposure to carbaryl. There was a small excess in the number of individuals with oligospermia (<20 million sperm/ml ejaculate) in the carbaryl workers, but the excess was not statistically significant. Reproductive hormone levels in the carbaryl exposed workers were normal.

Paradoxically when the semen samples collected by Whorton *et al.* (1979) were examined for abnormal sperm morphologies, a significant elevation of sperm abnormalities (abnormal head morphology) in currently exposed workers was detected (Wyrobeck *et al.,* 1981), but previously exposed workers did not exhibit significantly increased sperm anomalies. Certain of the study results, including a lack of correlation between the degree of exposure and the incidence of abnormalities and a negative correlation between years of exposure and the percentage of abnormal sperm confused a determination that carbaryl exposure resulted in abnormal sperm morphologies. The authors stated that, "With these data a definite link between carbaryl exposure and human seminal defects cannot be established". Similar concerns over the validity of the data to those presented above were expressed by the US EPA (1980) in a comment on the gonadal effects of carbaryl.

Furthermore, when duplicate slides to those examined by Wyrobeck et al. (1981) were examined via blind code by an independent examiner (McLeod, 1982) the following conclusions were obtained: 1) there was not an obvious depression of the sperm count in the exposed group; 2) the pattern of sperm morphology in both the treated and control group was "excellent"; and 3) there was no difference in the distribution of the sperm types. Statistical analyses of these findings confirmed the authors impressions (Hansen, 1982).

This reviewer concludes that carbaryl exposure does not increase the percentage of abnormal sperm or decrease sperm count in humans.

Leevitt *et al.* (1982) measured dermal and respiratory exposure as well as acetylcholinesterase activity in two groups of pesticide applicators spraying trees with carbaryl. They determined the mean dermal exposure to be 128 mg/hr in one group and 59 mg/hr in the second group. Respiratory exposures were reported to be 0.1 mg/hr in both groups. The maximum dose received by the workers was calculated to be 0.12%/hr of a toxic dose in group 1 and 0.02% of a toxic dose per hour in the second group. No overall inhibition of acetylcholinesterase activity was found in the applicators. The authors concluded "the use of carbaryl does not present a significant risk, in terms of acute toxicity, to professional applicators handling, mixing and applying this carbamate insecticide".

Thirty-eight part-time applicators were monitored in Nebraska for dermal and respiratory exposure (Gold *et al.*, 1982). The maximum dermal exposure was reported to be 2.9 mg/kg/hr; the maximum air concentration was 0.28 mg/m^3. The mean total exposure was calculated to be 0.01% of a toxic dose per hour. Paradoxically applicators treating trees had an average increase in acetylcholinesterase enzyme activity whereas applicators treating crops other than trees demonstrated no change or a slight decrease. The exposure rates were considered to be below that which could pose any risk or acute toxicity to urban applicators.

This reviewer concludes that occupational exposures should not and need not exceed the TLV. When the TLV is not exceeded there is no hazard to workers.

Poisoning in Humans

Symptoms of intoxication reported for man are the same as for other mammals and include miosis, excessive salivation, incoordination, epigastric pain, sweating, weakness, dizziness, shortness of breath, nausea, vomiting, hyperreflexia, pallor, nasal discharge, headache, tremors and dimness of vision (Hayes, 1963; Best and Murray, 1962; Long, 1971; Lopez, 1970; NIOSH, 1976).

Examples of intoxication with carbaryl appear in the literature which are instructive. Gastric lavage within half an hour after ingestion of an unknown amount of carbaryl by a 19-month-old infant was followed by a single 0.3-mg dose of atropine sulfate. Treatment was effective in controlling constriction of the pupils, salivation, and muscular incoordination. Recovery was apparently complete by 12 hours (Henson quoted by Best and Murray, 1962).

A drunken 39-year-old man swallowed approximately 500 ml of an 80% solution of carbaryl and was hospitalized within 1.5 hours confused but able to answer questions. Gastric lavage was performed and drugs to stimulate circulation were administered. The patient's condition deteriorated with poor vision and pulmonary edema. Atropine was given intravenously and intramuscularly at half hour intervals for a total dose of 6 mg with no sign of full atropinization. Three hours after ingestion of carbaryl, 250 mg of 2-PAM was administered. Pulmonary edema progressed rapidly, and the patient died 6 hours after ingestion. The patient would probably have recovered if more and only atropine had been administered. An empty stomach may have been a contributing factor speeding absorption. Alcohol probably played a role by slowing metabolism and increasing the magnitude and duration of exposure. The concentration of carbaryl reported in the blood, liver, kidney, and urine were 14, 29, 25, and 31 ppm, respectively (Faragó, 1969).

Hayes (1982) reported several poisoning and voluntary consumption cases, not available in the general literature, which provide insight into human response to excessive carbaryl exposure.

> A 250 mg dose of carbaryl was injested (approximately 2.8 mg/kg) by a male who after twenty minutes experienced a very sudden onset of violent epigastric pain followed by profuse sweating. A 1 mg dose of atropine produced little improvement and gradually lassitude developed and was followed by vomiting. One hour post exposure to carbaryl a total of 3 mg of atropine brought improvement and by 2 hours post exposure recovery was complete.

> An oral dose of 5.45 mg/kg carbaryl was injested by a man one hour and twenty minutes after a larger but undetermined dose had produced no symptoms. Five minutes after the 5.45 mg/kg dose he noticed a slight change in vision and difficulty in focusing that lasted 15 to 20 minutes. By 25 minutes post exposure, nausea and lightheadedness were experienced. Atropine, 2 mg, by mouth and reclining helped, but the symptoms returned. By 17 minutes after onset of nausea a total of 4.8 mg of atropine was administered. Sweating developed and clothing and the bedsheet became wet. Hyperperistalsis followed with remarkably loud bowel sounds in the near complete absence of pain. Nausea persisted for about 2 hours in the absence of vomiting and diarrhea. A profound sense of weakness was experienced with no difficulty in breathing. The sensorium remained completely clear, and questions were answered readily and correctly. Symptoms reached a maximum about 2 hours after onset. Pulse was near normal and the respiratory rate was 18 per minute. The pupils never became pinpoint, and there was no tearing, drooling, or rales. Definite improvement, including some increase in strength appeared in a little less than 3 more hours. Four hours after onset the subject was practically normal, walked about, and he ate a pint of ice cream. The subject retired, rested well and

experienced a normal day upon awakening including no difficulty with a full workload.

Additional information on human intoxication incidences is summarized in the EPA Carbaryl Decision Document (EPA, 1980). This document furnishes a summary of data obtained from reports of the Pesticide Incident Monitoring System (PIMS) from 1966-1980. For the period 1966-1980, 193 human intoxication cases were reported that involved carbaryl as the sole agent. These reports were categorized as 3 fatalities (suicides), 16 hospitalizations, 176 cases received medical attention, 124 cases that were affected but were not treated and 3 unaffected persons. The largest number of incidents involved home accidents and the second largest involved agricultural incidents.

Weston, Inc. (1982) concluded that the maximum number of deaths that could be related to carbaryl intoxication from PIMS was five. It is noteworthy that only one death, the suicide described earlier, is unambiguously attributable only to carbaryl.

Carbaryl has been used in enormous quantities under a variety of conditions and presents an enviable safety record. Accidental intoxications by adults are always survived. No delayed or irreversible effects due to acute intoxication or chronic exposures have been reported.

The medical department of Union Carbide maintains records on reports of human intoxication from carbaryl. Data on incidents between the years 1960 and 1976 were summarized by Harry (1977) and are reproduced below.

TABLE VI-1. Cases of Human Intoxication from Carbaryl (1960 - 1976).

Year	Probable Cases [a]	Unrelated Incidents [b]
1960	2	13
1961	6	5
1962	2	4
1963	1	19
1964	0	6
1965	0	0
1966	2	0
1967	0	5
1968	1	0
1969	1	3
1970	0	2
1971	3	5
1972	0	(several)[c]
1973	1	3
1974	1	5
1975	0	3
1976	0	7

[a]Cases in which there was exposure to carbaryl and resulting symptoms of illness were compatible with those expected from a cholinesterase inhibitor.

[b]Cases in which there was exposure to carbaryl but symptomatology was not compatible.

[c]Pakistan

Exposure Assessment

Carbaryl has been used for several decades as the agent of choice for numerous applications including: control of insects in citrus, stone and berry fruit, foroge, field and

vegetable crops, nuts, lawns, ornamental plants, rangeland, shade trees, poultry, and pets.

The U.S. Environmental Protection Agency (EPA) has established tolerances for carbaryl on food. Tolerances for residues of carbaryl (usually 10 ppm) have been established for 369 raw agricultural commodities. These tolerances are the maximum legal limit of carbaryl or its metabolites in or on commodities that enter commercial markets.

The agricultural use of carbaryl while theoretically possible is unlikely to lead to additional exposures via drinking water and aquatic food contamination. One pseudo-analytical approach to estimating dietary exposure to carbaryl from food is to consider the maximum allowable concentrations of carbaryl in or on each food commodity for which tolerances for carbaryl exist and to assume that all of these food items contain the maximum levels, and that no degradatioin occurs at any point in processing or cooking. Using these assumptions, the theoretical maximum exposure to carbaryl from the diet can be calculated by combining the data on tolerance levels on various foodstuffs with data on comsumption of these foodstuffs derived from the U. S. Department of Agriculture Food Consumption Survey of 1978-1979.

A quantitative analysis based on tolerances is the extreme case and not representative of actual dietary exposure. It would be completely illogical to assume that all 396 food items which can legally contain carbaryl would have been treated 100% of the time; that they contain the maximum legal residues in or on them; that there occurs no hydrolysis of the chemical in the environment after harvesting; and that no degradation of the chemical occurs during washing, blanching, or cooking processes.

The destruction of carbaryl by hydrolysis and food processing is a very important variable when estimating actual human exposures and will be discussed in detail. Quantative analysis of total dietary exposure to carbaryl is accomplished by multiplying actual or estimated food consumption times actual or estimated residue levels consumed. Such an analysis does not utilize the maximum allowable concentrations of the pesticide in or on each food commodity for which tolerances for carbaryl exist or assume that all food items contain the maximum levels (EPA-SAP, 1985).

There is ample evidence that maximum theoretical residue levels do not exist in food consumed by residents of the U.S. Results of the Food and Drug Administration's National Market Basket Surveys show that carbaryl residues in food at the market are found infrequently and then at levels well below the tolerance levels set for enforcement. This is as expected since carbaryl is susceptible to hydrolysis which is dependent on pH and temperature (Aly and El-Dib, 1971). The hydrolytic properties of carbaryl have been thoroughly researched (Union Carbide CBI, IRDC report, Nov. 9, 1976). Carbaryl is relatively stable at pH 3 at temperatures up to 35°C and pH 6 at 25°C. Carbaryl decomposes slowly at pH 6 and 35°C (half life of 29 days), and becomes increasingly unstable at high temperatures and higher pH. Carbaryl decomposes in approximately neutral river water within one week at 25°C (Eichelberger and Lichtenberg, 1971) and in sea water it is completely hydrolized after 17 days (Karinen, *et al.,* 1967). Exposure to carbaryl from underground water sources and food derived from river and sea waters is unlikely to occur. Carbaryl's hydrolysis is more rapid than other carbamate insecticides.

It is unlikely that substantial quantities of carbaryl could find it's way into man's diet via milk, flesh and eggs of animals consuming feed containing carbaryl residues. When dosed orally at the rate of 10 mg/kg, hens excreted approximately 50% of the carbonyl moiety as carbon dioxide within 48 hours. Ring-labeled activity was excreted mainly in the urine, with smaller amounts in the feces. Forty-eight hours after administration, 7.1% of radioactivity associated with the ring was retained in the body, whereas only 1.4% of activity associated with the carbonyl group was retained. Eggs collected for 12 days after dosing contained 0.33% of the ^{14}C administered (Paulson and Feil, 1969). Following oral intake of radioactive carbaryl ^{14}C labeled in the ring at dosages corresponding to dietary levels of 7, 21, and 70 ppm for 14 days, it was possible to recover 94.5, 0.153, and 0.05% of the total dose from the excrement, eggs, and carcass, respectively, and in proportion

to intake. The half-life was less than 1 day in the excrement and less than 3 days in the eggs. A metabolite tentatively identified as 1-naphthol sulfate, constituted 39.1% of the egg residues (Andrawes *et al.,* 1972).

It is unlikely that substantial quantities of carbaryl could occur as a residue in milk. Some of the intermediate metabolites of carbaryl are excreted in milk (Dorough and Casida, 1964). Following administration of carbaryl marked with ^{14}C-naphthol, radioactivity equivalent to about 0.35% of each dose was detected in the milk of cows. The highest concentrations (found in 6-hour samples) were 0.063 and 0.950 ppm following dosages of 0.25 and 3.05 mg/kg, respectively. The major chloroform-extractable metabolite was 5,6-dihydro-5,6-dihydroxycarbaryl. Radioactivity was last detected in milk collected 60 hours after administration. The proportions of the doses recovered in the urine and feces were 70 and 11%, respectively, at the lower dosage and 58 and 15%, respectively, at the higher dosage (Dorough, 1967).

Most of the carbonyl moiety of carbaryl that finds its way into milk is converted to lactose. When measurements were made using the compound tagged in the carbonyl group, about 1% of the original radioactivity was detected in the whole milk and about 0.9% in the milk after it had been skimmed. Of the material in skim milk, 87% was water-soluble, and 90 to 95% of this fraction was crystallized and shown to be lactose (Baron, 1968).

This reviewer does not believe milk, meat or eggs would be a source of carbaryl.

Even when commodities are eaten raw, they generally are washed reducing possible residue levels. Food processing practices such as blanching and cooking will dramatically reduce the carbaryl residues. Reductions in residue levels occur during both commercial processing and routine home preparations. Carbaryl is removed from green beans by washing (52 to 74% reduction) while cooking or blanching further reduces residues (50 to 81%) (Elkins *et al.,* 1968). Washing spinach with water decreased carbaryl residues by 66 to 88% while water blanching and cooking procedures further reduces the residue by 95 to 99% (Lamb *et al.,* 1968). Studies with residues on tomatoes and broccoli demonstrated similar results. Simple washing reduced carbaryl residue levels by 82 to 99% in tomatoes and 41 to 77% in broccoli (Farrow *et al.,* 1968,1969). Washing and cooking in commercial and home practice can be expected to reduce levels of carbaryl in commodities by a minimum of 58 to in excess of 99%. Since a high percentage of the average U.S. diet is composed of cooked vegetables and other cooked commodities, it is evident that the effect of processing significantly reduces the opportunity for exposure. This reviewer believes that a conservative estimate would be that average carbaryl residues in raw commodities is reduced at least ten fold prior to human consumption. Processing would be expected to produce an additional 10 fold reduction which in total represent a hundred fold decrease. Cooking and home preparation would reduce produce a further 10 fold decrease.

The importance of the reduction in commodity residue levels by processing is especially important when analyzing population sub-groups. For example, non-nursing infants less than one year of age is the population subgroup which would be projected to have the highest exposure to carbaryl if one utilized theoretical maximum residue values. The diet of this subgroup, however, is composed of highly processed grains, cooked and strained fruits and vegetables and juices from the pulp of citrus and pome fruits. Commercial baby food products are very highly processed and carefully monitored. Therefore, residues in the food of this group, which would have appeared to be the most heavily exposed, are reduced most significantly. Based on the probable effects of hydrolysis in the field and benefit of washing, cooking and processing the estimated levels of carbaryl in the diet is $1x10^{-4}$mg/carbaryl/kg/day.

The presence of carbaryl residues has been examined for in composite food samples collected for the Market Basket Surveys during the period from 1964-1973 (Duggan *et al.*, 1971; Duggan and Corneliussen, 1972; Manske and Corneliussen, 1974; Manske and Johnson, 1975; and Johnson and Manske, 1976). Only during 1964 did the daily

dietary intake (mg/kg body weight) for adults was estimated to reach a microgram level. In four of the nine years reported, daily intake was estimated to be zero. Daily intake ranges between 0.00001 mg/kg and 0.0005 mg/kg for the remaining four years. Average intake was 0.0002 mg/kg/day. The most recent published Market Basket Survey data covering the years 1976-1979 indicate that human exposure to carbaryl in food consumed ranged from undetectable to 0.000016 mg/kg/day (Gartrell *et al*, 1985). No reports exist linking adverse human health effects in the United States with residues of carbaryl in food.

Formation of N-nitrosocarbaryl is theoretically possible when foods containing carbaryl and nitrites are consumed simultaneously. The principle route of exposure to nitrite is via the consumption of foods, particularly cured meats, which have had nitrite added to enhance coloring or flavor or for product preservation. An additional source is via the reduction by salivary bacteria of ingested nitrate found particularly in fruits and vegetables. The National Academy of Sciences (1981) has estimated that average U.S. consumption of nitrite is 0.77 mg/person and that about 5% of the average consumption of nitrate (75 mg/person) is reduced to nitrite by the oral flora. This provides a total nitrite intake of 4.52 mg/person/day. The consumption of nitrite by non-nursing infants is very much lower but undetermined.

N-nitrosocarbaryl has been shown to be highly mutagenic in some *in vitro* systems but not *in vivo* (see III: Mutagenicity). N-nitrosocarbaryl has been tested as a pure compound and was found to be moderately carcinogenic. The opportunity for the administered carbaryl and endogenous nitrite ion to react in the milieu of gastric contents is inevitable and continuous during lifetime bioassays. Therefore, N-nitrosocarbaryl has been indirectly tested in all mammalian oral injestion bioassays of carbaryl (if N-nitrosocarbaryl is in fact formed *in vivo*) and has been found to be non carcinogenic (IV: Oncogenicity).

The following discussion will disclose those factors limiting the quantity of N-nitrosocarbaryl likely to be formed by humans as well as those influences on survival of N-nitrosocarbaryl after formation by nitrosation. N- nitrosocarbaryl is formed at an optimum pH of about 1.0. The pH is critical and only trace quantities of N-nitrosocarbaryl are formed at pH 2.0 and above. The toxicological significance of pH relative to N-nitrosocarbaryl synthesis is that the human stomach is very acidic, pH 1-2. The pH of the human stomach varies according to eating habits. The normal resting stomach ranges from pH 1 to 2 (Goldstein *et al.,* 1974; Grieve, 1961). Following a meal, the pH may increase to about pH 7, but returns to pH 1-2 within about 20 min (Grieve, 1961; Noller and Khodabakhah, 1964).

Elespuru *et al.* (1974) combined carbaryl with sodium nitrite in acid solution and obtained N-nitrosocarbaryl. Eleven percent (245 mg) of the theoretical yield of the nitroso derivative was formed when 0.5M concentration of carbaryl was reacted with a 2.0 M concentration of sodium nitrite in acetic acid (pH 2.9) for 1 hour at 37°C. Lesser yields were produced in hydrochloric acid and lower pHs. Yields of up to 72% were obtained by reacting carbaryl with sodium nitrite in acid under freezer conditions for 2 days (Elespuru, *et al.*, 1974).

Egert and Greim (1976) studied the effects of carbaryl in the presence of hydrochloric and acetic acid in the presence of excess nitrite for 4 hours at 37°C. A 67% yield of N-nitrosocarbaryl was reported.

Beraud *et al.* (1979) studied the formation of N-nitrosocarbaryl by the interaction of carbaryl with sodium nitrite in the gastric juices of the rat at 37°C. The concentration of N-nitrosocarbaryl increased for 45 minutes and then began to decline.

Rickard (1980) studied ^{14}C-labeled carbaryl *in vivo* nitrosation in the rat and guinea pig. Higher yields in the guinea pig were again attributed to lower pH (1.5) of the guinea pig than the rat (pH 3.5-5.5).

The optimum pH for the formation of N-nitrosocarbaryl is 1.0 and little is formed above pH 2.0 (Rickard *et al.* 1982).

Rickard and Dorough (1984) demonstrated the *in vivo* formation of N-nitrosocarbaryl in the stomach of rats and guinea pigs. When guinea pigs were given either simultaneous intubation of carbaryl (1 μmol) and sodium nitrite (1,160 μmol), or when these components were mixed in the guinea pig feed, approximately a 1.5% yield of N-nitrosocarbaryl was detected. Formation was very dependent on the amount of nitrite and the pH, and less dependent on the amount of carbaryl present. Increasing carbaryl from 0.025 to 2.5 μmol did not increase the percentage yield of the N-nitrosocarbaryl. The stomach pH (3.5-5.5) of the rat is higher than in guinea pigs (pH 1.5). A very low yield of nitrosocarbaryl was found in the rat (0.02%) at the same concentrations of nitrite and carbaryl. Carbaryl at 0.5 μM in aqueous HC1 at pH-1-2 and pH-3-4 was reacted with excess nitrite for 10 minutes at 37°C. A 42 to 64% yield was obtained at pH-1 while only trace amounts formed above pH-2. The half-life of N-nitrosocarbaryl decreased with decreasing pH. A half life of 25-34 minutes was obtained at pH 1.5.

Using ^{14}C-labeled carbaryl (Rickard and Dorough, 1984) attempted to isolate the N-nitrosocarbaryl from the stomach contents of rats and guinea pigs treated orally with the carbaryl and sodium nitrite. Only trace quantities of N-nitrosocarbaryl was recovered form the rat stomach, whereas 0.5 to 2.0% of the carbaryl dose was isolated as the nitroso derivative from the contents of the guinea pig stomach. The rather low apparent yields resulted in part from the instability of N-nitrosocarbaryl or possibly from absorption of the N-nitrosocarbamate. Higher rates of synthesis were indicated by incubating carbaryl with sodium nitrite in the presence of the stomach contents at 37°C for 15 min. About 30% nitrosation occurred with the guinea pig and about 0.5% with the rat. The difference was attributed to the pH of the gastric contents. For the rat, the pH ranged from 3 to 5 while gastric contents of the guinea pig had a pH between 1 and 2. Reaction of nitrite at 145 μM with carbaryl at 0.025, 0.25 and 2.5 μM produced a constant 0.1% yield of N-nitrosocarbaryl. It should be noted that 145 μM nitrite exceeds that expected in human adults. When nitrite was reduced to 7.5 μM yields dropped ten fold to 0.01%. Peak concentrations occurred at 30 minutes and by 120 minutes none could be detected.

The National Academy of Sciences (1981) estimated the average intake of nitrite is about .77 mg/person/day or about 5.6 μM per meal. An additional 3.4 μM is available from the conversion of nitrate to nitrite by the oral flora. A total of about 9 μM is available per meal. Rickard and Dorough determined that the yield of N-nitrosocarbaryl was greatly influenced by the concentration of nitrite in that 145 μM yielded a 0.1% conversion while 7.5 μM yielded 0.01%. The volume of the human stomach is at least 100 times that of the guinea pig used by Rickard and Dorough. Therefore it would be reasonable to assume at least a further tenfold reduction to 0.001%.

Conclusions

Carbaryl residue in human food is orders of magnitude below that required to produce any known toxicological effect.

In order to arrive at and justify the data to be utilized in Section VII regarding human cancer risk via the secondary formation of N-nitrosocarbaryl numerous uncertainties had tot be addressed. Consideration was given to the magnitude of exposure to carbaryl; subsequent conversion of ingested carbaryl to N-nitrosocarbaryl; the opportunity for any formed N-nitrosocarbaryl to reach target organs and molecules; and the relevance of the experimental evidence for estimating human risk. The magnitude and uncertainty inherent in assumptions necessary for the calculation, estimation or extrapolation to human cancer risk was carefully verified.

Predictions of N-nitrosocarbaryl levels based on the data of Rickard and Dorough (1984) are very likely to be overestimates of actual production in humans for many reasons. Experimental conditions producing N-nitrosocarbaryl were optimized because carbaryl and nitrite were introduced into the otherwise empty stomachs of guinea pigs. In

the human situation, carbaryl and nitrite would be present along with foodstuffs. Products of digestion would likely provide the opportunity for competing reactions to take place which would reduce the rate of carbaryl nitrosation. The foodstuffs in the stomach would also tend to increase the pH of the stomach which in turn would further reduce the reaction rate and speed spontaneous decomposition. Stomach contents would provide molecules with which the N-nitrosocarbaryl would react and inactivate the carcinogenic potential. The reactants necessary for production of N-nitrosocarbaryl would be introduced intermittently while eating rather than as a single bolus. The concentration of carbaryl and nitrite would be much lower than those studied in the experimental situation. Since the rate of nitrosation is strongly influenced by the concentration of the nitrite, it follows that the production of N-nitrosocarbaryl would likely be lower in the human situation. N-nitrosocarbaryl rapidly decomposes at pH 1 further decreasing the duration and quantity due to low level exposures of the reactants. N-nitrosocarbaryl would rapidly decompose at the more basic pH of the intestine.

VII: N-NITROSOCARBARYL CANCER RISK ANALYSIS

Summary

Potential for human exposure to N-nitrosocarbaryl, due to simultaneous dietary consumption of carbaryl and nitrite followed by nitrosation in the stomach, is theoretically possible but has not been documented. Exposures of $6x10^{-9}$ mg/kg/day of N-nitrosocarbaryl have been estimated by mathematical extrapolation. Even when worst case extrapolation models are used cancer risk is calculated to be below 1×10^{-6}. Risk levels below 1×10^{-6} calculated by this method are not of concern to regulatory agenices. The risks calculated, using more likely assumptions and reassonable mathematical models are vanishingly small and in the order of $1x10^{-9}$ - $1x10^{-12}$. Concentrations of nitrite and carbaryl required to obtain experimentally carcinogenic levels of N-nitrosocarbaryl by *in vivo* nitrosation cannot be obtained since they exceed the lethal levels of carbaryl and nitrite by several orders of magnitude.

Hazard Indentification

The safety of carbaryl with respect to oncogenicity has been well documented (see IV: Oncogenicity) and would seem to provide overwhelming confidence that pesticidal efficacy and negligible human exposure justify continued use. Carbaryl however is a secondary amide and therefore capable of nitrosation when the hydrogen atom of the amide group is replaced by a -N=O group to form a nitroso derivative. Carbaryl is a precursor of N-nitrosocarbaryl, a modestly carcinogenic substance in the rat The human diet and saliva contains nitrite, the source of -N=O. The contents of the human stomach are acidic, a condition that is necessary for the nitrosation reaction to proceed. Carbaryl has been nitrosated in several *in vitro* studies, and of special note, in the stomach of guinea pigs whose stomachs acidity is comparable to that of the human.

All studies which jointly administered nitrite and carbaryl have yielded negative results for oncogenicity. Studies conducted on N-nitrosocarbaryl synthesized *in vitro* are all that are available for extrapolation. The oncogenicity of N-nitrosocarbaryl was reviewed in detail in IV: Oncogenicity. A short summary follows for the convenience of the reader.

The potential of N-nitrosocarbaryl to be formed in oncogenic quantities by *in vivo* nitrosation of carbaryl and nitrite in the stomach of rats was studied by Lijinsky and Taylor (1978). Tumor production in treated female pregnant rats and their subsequent offspring was considered a sensitive indicator of both *in vivo* and *in utero* carcinogenic potential.

Any N-nitrosocarbaryl formed in the stomach of the dams could possibly be transported transplacentally to fetuses. The dosages utilized was 600 mg carbaryl/kg and 800 mg nitrite/kg. At these doses the nitrite was fatally toxic to some of the pregnant rats. The results led the investigators to conclude that there was not an excess of tumors in the treated animals. The direct quantitative comparison of this study to production of N-nitrosocarbaryl in humans is not appropriate since less nitrosation will occur at the relatively high pH of the rat stomach (*i.e.,* 3 to 5). Reduced reaction rates are more than compensated for however since the reactants were administered at about 1,000,000 times the expected human exposure.

Studies administering carbaryl and nitrite are negative. Hazard identification is therefore replaced by risk estimation and preformed N-nitrosocarbaryl studies are all that are available. The N-nitrosocarbaryl carcinogencity study most useful for risk estimation is that by Preussman *et al.* (1976) in which rats received N-nitrosocarbaryl by gavage twice weekly for their lifetimes. For the purpose of subsequent extrapolation exercises, the twice weekly dose of 130 mg/kg must be adjusted to be equivalent to a daily dose (130 x 2/7 mg/kg = 37.14 mg/kg/day). At this dose level, the incidence of stomach tumors was 17/32 compared to none in the controls. The data of Linjinski and Taylor (1976) for female rats will be used later when performing worst case calculations with the one-hit linear model.

Low Dose Risk Assessment

Determination of the relationship between the magnitude of possible exposures to N-nitrosocarbaryl and the probability that such an exposure will result in the occurrence of cancer constitutes dose-response risk assessment. The limitations of such extrapolation-toxicological evaluations should be stated clearly. It was not, is not and will not be possible to guarantee absolute safety (Cranmer, 1974). Small populations of experimental subjects, either animal or man, provide an imprecise estimate of hazard for comparison to a large human population of variable genetic/disease states, cultural backgrounds and ages.

When direct measurements are not available, as in the case of N-nitrosocarbaryl, extrapolation must be made. Extrapolation of cancer hazards to humans is based on animal studies conducted at relatively high and constant dose rates wihile humans are exposed at intermittently to variable and low dose rates. One task to be performed by the extrapolator is the selection of descriptors of the function relating hazard as measured at high dose rates to theoretically estimated animal risk at low dose rates. The magnitude of low dose risk is extremely dependent upon the assumed shape of this dose-response function (Crump *et al.,* 1976). Unfortunately this shape at low dose rates is more sensitive to manipulating the extrapolation procedure than to the experimental data. This has led this reviewer to the discouraging observation that extensive experimentation and quality assurance means less than the arbitrary selection of mathematical descriptors selection or manipulation.

The extreme differences between models for extrapolating to low risks have been reviewed in detail elsewhere (Cranmer, 1974, 1977a, 1977b, 1981). A simple comparison of three models which can be used for calculating risk for N-nitrosocarbaryl is presented in Table VII-1. The probit, logistic, and one-hit curves all equally predict a 50% tumor response at a unit dose and 16% tumor response at 1/4 that unit dose. The families of curves generated by these models are indistinguishable in the 8% to 92% tumor response range. Several thousand animals would be required to distinguish between the probit and logistic curves in the 2% to 4% response range with no guarantee that either model would be applicable at lower levels. Extreme differences between models in estimated doses are noted when extrapolating to a 1 in a million or a 1 in 100 million risk.

TABLE VII-1. Quantity of N-Nitrosocarbaryl (mg/kg/day) Required to Produce Various Lifetime Cancer Risks According to Three Mathematical Models.

Cancer Lifetime Risk[a]	Mathematical Model				
	Probiit			Logistic	One Hit
	1.0[b]	1.5[b]	2.0[b]		
10^{-3}	$6x10^{-1}$	$2x10^{0}$	$5x10^{0}$	$1x10^{-1}$	$6x10^{-2}$
10^{-6}	$6x10^{-2}$	$2x10^{-1}$	$1x10^{0}$	$6x10^{-4}$	$6x10^{-5}$
10^{-8}	$2x10^{-2}$	$6x10^{-2}$	$3x10^{-1}$	$4x10^{-6}$	$6x10^{-7}$

[a]Upper 99% Confidence Level
[b]Slope

A few comments on "threshold" are an appropriate prelude to our discussion of several of the many methods available for mathematical extrapolation. The concept of a threshold dose is based on the premise that some smaller dose under similar conditions will not produce the measured effect. If the conditions change there will be a change in the threshold. This does not mean thresholds don't exist! This does not mean that thresholds can't be estimated! It does not mean that bounds cannot be established! It means only that thresholds just as all other phenomena in toxicology are not absolute. There is no mathematical reason to set cancer aside as different from other toxicological effects. In the absence of a threshold a no-effect dose for any effect not just cancer, is sample size dependent.

Several calculations of risk to N-nitrosocarbaryl will be offered in Table VII-3. The following discussion is offered to explain some of the assumptions inherent in the use of the various models. Emphasis is placed on models used by EPA in pesticide regulation.

Crump *et al.*(1976), Guess *et al.* (1977) and Peto (1978) have invoked certain hypotheses of carcinogenesis, to argue that there exists the possibility of linear responses at low doses. For example Crump *et al.* (1976) and Guess *et al.* (1977) offer the proposition that the probability P(d) of response at dose rate is given by

$$P(d) = 1\text{-}\exp[-(a_0 + a_1 d + a_2 d^2 + \ldots)],\ a_i \geq 0.$$

When P(d) is small, the excess risk P(d)-P(0) is approximately

$$P(d)\text{-}P(0) = a_1 d + a_2 d^2 + \ldots,$$

and the question of nonthreshold low-dose linearity is determined by whether or not the coefficient a is zero. There are plausible assumptions, such as detoxification mechanisms, DNA repair, or the necessity for multiple molecular interactions, that lead to $a_1 = 0$. There are also plausible assumptions for some agents that can lead to positive values for a_1. We will never have the knowledge to completely discriminate between these alternatives. The most acceptable approach is to use extrapolation procedures that make effecient use of what we have or can get, the experimental data. Substitution of upper confidence limits in place of central expected values for the coefficients a_i incorporates a bias value judgement into the extrapolation procedures that almost always outweighs the utility of the data or the model (see Table VII-4). When one applies upper confidence limits rather than central expected value for a_1, it is equivalent to accepting that a_1 is positive. At low dose rates this value judgement (an arbitrary upper confidence limit) dominates the remaining terms. The requirement of the use of upper confidence limits rather than central expected value guarantees that certain models will yield nonthreshold linearity at low dose rates.

The lack of logic to support this premise becomes apparent when one examines the procedures of Crump *et al. (1976)* and Guess *et al.* (1977) since the techniques become so insensitive to response at experimental dosages that they fail to distinguish between low levels of potent carcinogens and noncarcinogenic substances. Guess *et al.* (1977) have documented this unacceptable paradox by using the comparison of two dose response data sets for a noncarcinogenic agent. One simulation involved 150 responses at each of ten dose rates. The second involved 300 responses at each of 5 dose rates. In both cases the 90% upper confidence limit on a_1 was positive, so that the two upper confidence curves relating extra response to dose rate were virtually linear at low dose rates. The guarantee of false positive risk estimates support this reviewer's contention that the exclusive use of models requiring upper confidence limits is not acceptable.

Another example of mathematical mischief is provided by Gaylor and Kodell (1980) in their study of 14 sets of toxicological dose response data previously described by the Scientific Committee of the Food Safety Council (1978). The behavior of a gamma multihit model was compared to the Armitage-Doll multistage model for low dose extrapolation using the technique given by Crump *et al.* (1977). The Gaylor and Kodell Armitage-Doll multi-stage model results do not exactly agree with the Scientific Committee of the Food Safety Council (Wodicka *et al.,* 1978) because the limits calculated by Gaylor and Kodell did not assume a maximum degree of dose in the exponential term, whereas the limits calculated by the Food Safety Council took the degree of dose as fixed. Gaylor and Kodell (1980) concluded "Conceptually linear extrapolation would (should) be more conservative, but this is not the case in actuality apparently because more stringent mathematical assumptions and conditions are required" (by the gamma one-hit model). Selected examples are given in Table VII-2.

Table VII-2. Lower 97.5% Confidence Limit for Dosages Predicted to Have a Risk of Less Than One Million in Rodent Test Populations.

Substance	Dose Unit	Sci. Com.[a] FSC	Linear	Armitage-Doll
Aflatoxin B1	ppb	3.4×10^{-5}	7.9×10^{-6}	5.9×10^{-6}
Vinyl Chloride	ppm	1.6×10^{-3}	7.1×10^{-4}	5.2×19^{-4}
Ethylenethiourea	ppm	4.4×10^{-4}	1.0×10^{-4}	3.2×10^{-4}
Dieldrin	ppm	9.4×10^{-6}	5.7×10^{-6}	2.9×10^{-6}
DDT	ppm	2.0×10^{-2}	3.4×10^{-5}	2.6×10^{-5}

[a]Scientific Committee of the Food Safety Council.

If one wishes to embrace the assumption that some risk of cancer results from even a vanishingly small exposure to a carcinogen, the exponential probability law can be applied to extenuating response at various exposures.

$$P_{(d)} = 1\text{-}e^{(-\lambda d)}$$

The probability law predicts the probability, P, that a carcinogen at a dosage, d, will induce a tumor. Some of the tumors in the treatment group will be spontaneous and not induced by the carcinogen. Where P_c is the probability that a tumor develops spontaneously in a control group and P_t is the probability that a tumor develops in the treatment group, the probability that a tumor does not develop in the treatment group was shown by Abbott (1925) to be $(1 - P_t) = (1 - P_c)\ (1 - P)$

$$\text{Solving, } P = \frac{P_t - P_c}{1 - P_c}$$

Substituting gives the probability of observing a tumor in the treatment group,

$$P_t = 1 - (1 - P_c)e^{(-\lambda d)}$$

$$\text{Solving, } P = \frac{1}{d} \ln (1 - P_t)/(1 - P_c).$$

At very low dosages, P is almost directly proportional to the dosage d and λ will be the slope of the dose-response curve obtained when P is plotted against values of d close to zero. Because this form of the one-hit model approximates a straight line through the origin at very low dosages, it is often referred to as the linear non-threshold dose-response model. Some have place great faith in this mathematical paper exercise as "proof" that any exposure to a carcinogenic substance will result in some risk of tumor incidence, and that the risk or probability of cancer induction at low dosages must increase linearly with increasing dose.

Another variation of the linear extrapolation procedure derives dose-response curves from a computer based one-hit model developed by Dr. Charles Brown while at the Biometry Branch, Division of Cancer Cause and Prevention, National Cancer Institute. The mathematics of the Brown one-hit model differ from the one-hit model in that Brown's model takes into account the tumor incidences at all dietary concentrations simultaneously without regard to the statistical significance of any. The computer program for the Brown model also allows derivation of 95% and 99% upper confidence limits of λ regardless of the form of the one-hit model used.

The larger the value or the steeper the dose-response curve at low-dosages. By either the one-hit model or the Brown model, the values of λ will be consistently less for one sex than the values similarly derived using data from the other sex. Some have elected to incorporate an additional measure of "conservatism", by utilizing the most sensitive data (study, sex, strain, route, duration, species) for all calculations involved in the derivation of final estimates of carcinogenic risk.The second stage used by EPA in the derivation of estimates of carcinogenic risks involves the transformations of each study from experimental animal to a λ for humans. This transformation is accomplished by the application of a species conversion factor. This species conversion factor is based upon the EPA stated knowledge that the specific dose in mg/kg/day of a direct acting agent required to produce an effect in humans is smaller than the specific dose of that agent that would be required to produce a similar effect in a given animal species. According to EPA, if dietary concentrations or exposure levels are expressed in mg/kg/day, then the potency of a chemical or direct-acting drug in humans is higher than in animal by the ratio:

$$[(\text{Average human weight})/(\text{Average animal weight})]^{1/3}$$

If the average human weight is assumed to 70kg (70,000g) and the average weight of a rat is assumed to be 350g, then the conversion factor is $(70{,}000g/350g)^{1/3} = (200)^{1/3} = 5.85$ (Anderson, 1979).

Each point estimate of the value λ from the data, along with associated 95% and 99% upper confidence limits on λ is multiplied by the species conversion factor to yield a point estimate of the value of λ for humans along with associated 95% and 99% upper confidence limits. To arrive at final quantitative estimates of carcinogenic risk from potential human exposures, each point estimate of λ and associated upper 95% confidence limit is combined with the range of exposure estimates, d, and applied to

$$P(d) = 1 - e^{(-\lambda d)}$$

the exponential probability law. After performing these calculations EPA states "The respective resultant values of P are the point estimates and upper 95% and 99% confidence limits of risk or probability that a lifetime of human exposure at dosage level, d, would induce a tumor in a human so exposed."

Table VII-3 reviews the data available for N-nitrosocarbaryl. All subsequent calculations are rounded to the nearest whole number. Rounding to the nearest power of ten is probably justified by the significant uncertainty of the data.

Table VII-3. Estimates of Unit Cancer Risk (UCR) for N-Nitrosocarbaryl.

Study	Average Lifetime Dose	Excess Tumor Indicies	Estimate of UCR
Preusman *et al* (1976)	37.1	17/32	0.020
Lijinsky et al (1976)	1.1	21/28	1.26

Table VII-4 has been constructed to indicate risks associated with various lifetime exposures to N-nitrosocarbaryl on the two experiments available and the unit cancer risk.

Table VII-4. Estimates of Potential Risks to Humans from N-Nitrosocarbaryl $P(d) = 1 - e^{(-\lambda d)}$.

	Worst Case	Reasonable Estimate	Monitoring
Exposure (mg/kg/day)	$1x10^{-6}$	$1x10^{-10}$	$1x10^{-10}$
Preussman *et al.*	$2x10^{-7}$	$2x10^{-10}$	$2x10^{-11}$
Lijinsky *et al* (1976)	$1x10^{-6}$	$1x10^{-9}$	$1x10^{-10}$

NCTR's Gaylor and Kodel (1980) in explaining their technique of linear extrapolation (interpolation) and relying on ED_{01} for support state "The development of more sophisticated mathematical models to extrapolate to low doses with quantal bioassay data does not appear to be justified". Their statements seem reasonable for limited data bases as is demonstrated for N-nitrosocarbaryl in Table VII-4 where models which are forced linear at low dosages yield the same answer.

Gaylor and Kodell have suggested a linear interpolation technique which is described mathematically over the low unobservable dose range by

$$P = \frac{UCL}{d_e} \times d$$

where P is the upper bound on the potential proportion of animals with excess tumors caused by the administration of a dosage, d, of a chemical and UCL is the upper confidence limit at a dosage, d_e, in the experimental dose range. Linear interpolation is suggested for use between zero and the upper confidence limit of the excess tumor rate estimated for the lowest experimental dosage. Any mathematical model can be used which adequately fits the data in the experimental dose range since it makes relatively little difference on the resultant value. The only purpose of this model is to obtain an upper

confidence line for the experimental region. The fitted line from the selected model is not extended below the experimental region.

Additional hazard from cancer accrues to those additional people who might eventually contract cancer. Cancers, created by low doses of carcinogens in experimental animals generally occur late in life and are not lethal. The impact of these cancers is most appropriately measured as a reduction of quality of remaining life. Murray and Axtell (1974) were among the first to investigate the utility of time-to-tumor models for extrapolation. The simplest carcinogensis bioassay protocol which can be employed to access the dynamics of tumor development measures tumors in animals sacrificed at fixed points in time and observes the percentages of those animals possessing particular tumors. This sampling technique yields estimates of the proportion with tumors at given times which can then be plotted against dose. A disadvantage of such experiments is that they usually require large numbers of animals. No experiment is available for N-nitrosocarbaryl which permits application of time-to-tumor models.

Research in statistical techniques to analyze time-to-tumor data continues. One useful measure of the impact of carcinogenesis on a population is age-specific incidence rates of the amount of life shortening due to a tumor. Time-to-tumor studies do not, as often suggested, require life time data to estimate a relationship between tumor rates, time and dosage. Indeed extrapolations to low dosages often project values for median time to tumor beyond the normal lifespan of an animal. Time-to-tumor distributions must however estimate the proportion of animals expected to develop tumors within their lifespan before dying of other causes. Approaches tend to be complicated and, just as all models do, depend on mathematical factors subject to bias. Relevant experiments simply require survival and tumor data at several dosages but unfortunately are not available for N-nitrosocarbaryl.

Some debate exists regarding the most appropriate units of daily dose to use when presenting data to be modeled. As discussed earlier, EPA normally employs units adjusted for differences in body surface area per unit mass. Units of mg/kg/body weight/day however seem to give the best correlation between carcinogenicity in rodents and humans (Crump *et al.*). Mg/kg/body weight is the unit used in the examples presented except in the case of the one hit linear model which conforms to EPA criteria and the cancer data of Linjinski and Taylor (1976) for female rats. The values of risk calculated for all other examples are those calculated form the data of Preussman *et al.* (1976) for the male rat at an exposure of 6×10^{-9} mg/kg/day. In the absence of data to the contrary it is assumed that rats and humans are at the same risk when exposed to the same daily dose level for their respective lifetime.

A short summary follows. Mathematics are used to predict incidence of cancer expected to be associated with any particular level of exposure. Experimental doses in the case of N-nitrosocarbaryl are many orders of magnitude greater than those to which humans are exposed. Much controversy has surrounded the shape of dose response curves outside the observation range. The slope of the dose response curve as well as thresholds are greatly influenced by numerous biological processes including the individual's ability to scavenge reactive metabolites and repair damage to cellular components. The site of carcinogenic action of N-nitrosocarbaryl is site of first tissue contact. Distribution through the body and biotransformation probably does not play a confounding role in influencing the slope of a dose response curve for N-nitrosocarbaryl. A moderate slope of 2 has been selected for calculations performed with the models presented. The carcinogencity studies available for comparison and manipulation employed only one dose level and only negative data exist for humans. It is still possible however to use extrapolation models such as the multistage, multihit or Weibull to estimate the risk at low-dose levels by selecting reasonable slope functions.

Conclusions

If N-nitrosocarbamates, (N-nitrosocarbaryl may or may not be produced in humans) are a real problem, it is likely be greatest for those individuals consuming prescribed carbamate-type drugs. If N-nitrosocarbamates are indeed formed in the stomach of the general population, the incidence of stomach cancer might have been expected to increase over the past 20 years since that period has seen heavy carbamate insecticide and drug use. The incidence of gastric cancer in the United States has actually declined substantially during this period (Weisburger and Raineri, 1975). Carbaryl insecticide simply is not present in the diet of the vast majority of people for the vast majority of the time. It is also unlikely that when residues do occur that large excesses of nitrite would also be present, that the stomach pH would be optimum for nitrosation or that formed N-nitrosocarbamate would not first react with foodstuffs being digested. Even if we assume that exposures of $6x10^{-8}$ mg/kg/day occurs, any risk calculated for such an exposure will be vanishingly small.

Table VII-5. Extrapolation Methods for Risk[a] to 6 x 10^{-9} mg/kg/day N-Nitrosocarbaryl.

Model	Formula	Risk[b]
Probit Function (point exposure)	$f_a = 1 - \exp[-QD]^*$	$<1x10^{-9}$
Log-Probit Model	$P_{(d)} = \Phi(\alpha+\beta\log^{10}d)^*$	$<1x10^{-9}$
Power Law	$f_a = bd^{k^*}$	$<1x10^{-9}$
Doll	$I_t = b(t-v-w)^k$ †††	-
Doll-Weibull	$I = bd^m(t-w)^k$ †††	-
One Hit Linear †	$P_{(d)} = 1-e^{(-\lambda d)}$ ††	1×10^{-6}
Extreme Value †	$P_{(d)} = 1-\exp[-\exp(a + \beta\log d)]$	1×10^{-7}
Linear Interpolation †	$P_{de} = UCL \times d$	1×10^{-7}
Multi Stage †	$P_{(d)} = 1-\exp[-(a + B_1 d) \ldots (\lambda_k + \beta_k d)]$	1×10^{-7}
Multi-hit (k-hit) Model †	$P_{(d)} = 1 - \Sigma^{k-1} (\lambda d)^{ie-\lambda d/i}$	1×10^{-7}
Median Effect Principle of the Mass-action Law	$f_a/(1 - f_a) = (D/D_m)^m$ †††	-
Chou Equation	$fa = [1 + (D_m/D)^m]^{-1}$ †††	-

[a] Risks calculated as a 99% probability that not one excess cancer will develop, in the population of the United States over a life time for static models or in the next 100 years for time to tumor modesl, are considered to be 0.

[b] Risk is rounded to the nearest whole number.

* An arbitrary slope function of 2 was selected.

† Conservative assumptions force apparent variables to become constants for single dose experiments.

†† Data of Linjinsky and Taylor (1976)

††† Data insufficient for model use.

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