Chapter 74

Toxicological Profile of Carbaryl

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74.1 INTRODUCTION

Carbaryl is a carbamate pesticide that was first registered for use in the United States on cotton in 1959. Today, carbaryl is a widely used broad-spectrum insecticide used in agriculture, professional turf management, ornamental production, and residential settings. Carbaryl is sold under many trade names, but the most common one is Sevin. Over the years, a large dossier of toxicity, environmental fate, residue, and monitoring data have been generated, which, coupled with practical use experience, have been used to improve application methods and to refine exposure and risk assessments that support the continued registration and safe use of this insecticide.

74.2 DESCRIPTION, USE, AND BIOLOGICAL MODE OF ACTION

Technical carbaryl belongs to the *N*-methyl carbamate chemical family, which act via inhibition of acetylcholinesterase. The pure (technical) material is a white to light tan solid with a water solubility of approximately 40 ppm at 25°C. The empirical formula of carbaryl is $C_{12}H_{11}NO_2$, with a molecular weight of 201.2. Its Chemical Abstract Service (CAS) name is 1-naphthalenyl methylcarbamate and its CAS number is 63-25-2.

Carbaryl end-use products are formulated as flowable concentrates, granules, wettable powders, baits, dusts, and ready-to-use sprays. Application methods include groundboom, airblast, chemigation, aerial, drop or broadcast granular spreaders, and handheld equipment such as low-pressure handwand sprayers.

74.3 HAZARD CHARACTERIZATION

Carbaryl has a very robust toxicity database including acute, chronic, developmental, reproductive, and neurotoxicity studies. The toxicity data summarized in this chapter come from studies conducted during the past 20 years to support the registered and approved uses of carbaryl. A review of older toxicity data can be found in Cranmer (1986), while more recently, Gunasekara *et al.* (2008) reviewed the environmental fate and toxicology of carbaryl.

74.3.1 Acute Toxicity

Carbaryl is moderately toxic by the oral route and exhibits a low level of toxicity following dermal or inhalation exposures. In recent acute toxicity studies, the oral LD50 in rats was 614 mg/kg [vehicle of aqueous 0.5% (w/v) carboxymethylcellulose] while the dermal LD50 in rats was greater than 5000 mg/kg (Griffon, 2001a,b). An acute inhalation (nose only) exposure study in rats with carbaryl resulted in LC50 values of greater than 4.62 mg/l in males and 2.43 mg/l in females (Wesson, 2001). Carbaryl is not a dermal or eye irritant and is not a dermal sensitizer in guinea pigs (Griffon, 2001c,d,e).

Rapid and reversible cholinesterase inhibition (ChEI) has been demonstrated following acute oral exposure in rats (Brooks and Broxup, 1995a). A dose-dependent inhibition of brain and red blood cell (RBC) ChE activity was observed 30 min after oral administration of carbaryl suspended in aqueous 0.5% (w/v) carboxymethylcellulose/0.1% (w/v) Tween 80. Thereafter, the level of inhibition observed at 10 and 50 mg/kg declined slowly and returned to control levels by 24 h, but at 125 mg/kg, ChE activity remained slightly below control levels at 24 h after dosing. The results for male rats are summarized in Table 74.1 and are similar to those observed for female rats.

Observed clinical signs and behavioral changes were also dose-dependent and consisted of a single observation of muzzle and urogenital staining in one male at 10 mg/kg, and tremors, salivation, muzzle and urinary staining, gait alterations, decreased respiration, and decreased activity

TABLE 74.1 Time Course of ChEI in Male Rats Receiving a Single Oral Bolus Dose of Carbaryl

Time after dosing	RBC (% control)			Brain (% control)		
	10 mg/kg	50 mg/kg	125 mg/kg	10 mg/kg	50 mg/kg	125 mg/kg
0.5 h	72	68	56	46	23	18
1.0 h	82	71	54	68	25	22
2.0 h	88	65	71	82	33	21
4.0 h	84	63	51	94	52	22
8.0 h	101	100	56	91	73	24
24 h	103	94	90	100	94	77

and arousal levels at 50 and 125 mg/kg. The incidence and severity of these signs decreased during the 24-h period after dosing. In another study, oral administration of carbaryl in corn oil at 30 mg/kg produced a 40% inhibition of both brain and RBC ChE activity 30 min after dosing (Padilla *et al.*, 2007). Two hours after dosing, recovery of ChE activity to near control levels was observed for both brain and RBC, but a second phase of inhibition was noted for the RBC matrix at the 4- and 6-h time points with return to control levels by 24 h after dosing.

Dermal absorption studies in rats with different formulated products show that carbaryl has a low rate of penetration. When applied to the shaved back of male rats, the mean percent of radioactivity absorbed after a 10-h exposure was 12.7 and 8.9 for a 100-fold dilution in 1.0% aqueous carboxymethyl cellulose of Sevin brand LXR Plus (a liquid formulation containing 44.1% active ingredient) and Sevin brand 80S (a dry powder formulation containing 80% active ingredient), respectively (Cheng, 1994, 1995).

74.3.2 Subacute/Subchronic Toxicity

The subchronic toxicity of carbaryl has been assessed in rats, mice, and dogs. In 6- to 8-week dietary studies, a decrease of body weight and food consumption was observed at dietary levels of 3000 ppm and higher in Sprague-Dawley Crl:CD BR rats and at 4000 ppm and higher in CD-1 mice (Hamada, 1990a, b). RBC and brain ChE were decreased at 3000 ppm and higher in both rats and mice. Increased liver weight associated with centrilobular hypertrophy was observed at 6000 ppm in rats, whereas centrilobular hypertrophy without increased liver weight was noted in mice at 4000 ppm. In a 6-month dietary study in C57Bl/6 male mice, carbaryl administered at 4000 ppm resulted in slightly lower body weights and food consumption and a 14% increase in absolute liver weight without any associated histopathological changes (Chuzel, 2000a).

In a 5-week study, dogs were administered carbaryl via the diet at 0, 20, 45, and 125 ppm (Hamada, 1991). No evidence of treatment-related effects was noted for the parameters measured in this study including RBC and brain ChE. In a 1-year study conducted with dietary levels of 0, 125, 400, and 1250 ppm, clinical signs of toxicity (lacrimation, salivation, and tremors) and lower body weight gain and food consumption were observed in female dogs at 1250 ppm (Hamada, 1987). RBC ChE was significantly decreased at 400 ppm during the first half of the study and at 1250 ppm throughout the entire study. At study termination, brain ChE was 22 to 36% lower than control at 400 and 1250 ppm (both sexes) and 20% below control at 125 ppm in females. No histopathological changes related to treatment were observed.

In a 4-week study, carbaryl was administered by topical application to the shaved dorsal skin of rats for 6 h/day, 5 days/week at 0, 20, 50, or 100 mg/kg/day (Austin, 2002). Lower body weight gains were observed at 100 mg/kg/day, but no treatment-related findings on mortality, clinical signs, food consumption, or dermal irritation were noted. RBC ChE, determined during each week of the study within 1 h after removal of the test material, was 10 to 20% lower than control at 50 and 100 mg/kg/day with no clear dose response. At study termination, brain ChE at these dose levels was 15 to 24% lower than control. Benchmark dose (BMD) analysis of the brain ChE data to determine a benchmark response of 10% gives a BMD_{10} of 49.35 mg/kg/day with a 95% lower confidence limit ($BMDL_{10}$) of 30.56 mg/kg/day (US EPA, 2008).

74.3.3 Neurotoxicity

There is a complete neurotoxicity database on carbaryl consisting of acute, subchronic, and developmental neurotoxicity studies conducted with Sprague-Dawley rats. Based on acute neurotoxicity testing in rats with exposure to carbaryl via oral gavage, the highest nonlethal dose was

determined to be 250 mg/kg (Brooks and Broxup, 1995b). In an acute neurotoxicity study with groups of rats dosed by oral gavage at 0, 10, 50, or 125 mg/kg, dose-related alterations in the functional observation battery (FOB) were observed 30 min after dosing (Brooks and Broxup, 1995c). FOB alterations included ataxic gait in one male and one female at 10 mg/kg and tremors; increased salivation; decreased or impaired locomotor activity, rearing, gait, arousal, and reflexes; and decreased body temperature at 50 and 125 mg/kg. In addition, increased hindlimb splay and startle response and decreased grip strength were seen at 125 mg/kg. Lower motor activity counts were observed approximately 1 h postdosing for all treated groups. No treatment-related alterations in FOB or motor activity were observed 7 or 14 days after dosing. Further, no neuropathological lesions were observed at study termination.

The subchronic neurotoxicity of carbaryl was evaluated over a 13-week exposure period in rats by oral gavage at 0, 1, 10, and 30 mg/kg/day (Robinson and Broxup, 1996). Body weight and food consumption were decreased at 30 mg/kg/day throughout most of the study. Neurotoxicological evaluations conducted during weeks 4, 8, and 13 at approximately the time to peak effect, that is, between 0.5 and 1 h postdosing, revealed clinical signs and FOB changes such as increased salivation, tremors, pinpoint pupils, gait alterations, decreased rearing, reduced grip strength, as well as reduced body temperatures at 30 mg/kg/day and to a lesser extent at 10 mg/kg/day. The motor activity counts were also reduced at 30 mg/kg/day in animals at weeks 4 and 8. Inhibition of RBC and brain ChE was observed 1 h postdosing for samples collected during weeks 4, 8, and 13. At the end of the exposure period, no treatment-related neuropathological findings were observed. The no observed adverse effect level (NOAEL) was established at 1 mg/kg/day based on ChEI and alterations in the FOB.

A developmental neurotoxicity study was also conducted by oral gavage at 0, 0.1, 1, and 10 mg/kg/day (Robinson and Broxup, 1997). Pregnant rats (F0) were treated from gestation day 6 (GD6) to lactation day 10 (LD10). Pups (F1) were weaned on postnatal day 21 (PND21) and observed up to PND70. Maternal performance parameters such as pregnancy rate, gestation index, sex ratio, and implantation loss were unaffected by treatment. In F0 dams at 10 mg/kg/day, tremors, reduced pupil size, and altered gait were noted during the dosing period, and RBC and brain ChE were significantly decreased at 1 h after dosing on GD20 and LD10. No neuropathological changes were observed at terminal sacrifice.

For the F1 generation, no in-life phase parameters were affected including survival, body weight, clinical signs, FOB, motor activity, developmental landmarks, auditory startle, passive avoidance, and water maze measurements. Further, no changes in brain weight or neuropathology were observed at PND11 or 70. Extensive morphometric measurements showed no clear treatment-related effect at 10 mg/kg/day. Therefore, based on observations in the FOB and on ChEI, the NOAEL for maternal toxicity was set at 1 mg/kg/day. As no treatment-related effects were observed in pups, the NOAEL for developmental neurotoxicity in the offspring was 10 mg/kg/day, the highest dose level tested.

In conclusion, across all of the neurotoxicity studies, the most sensitive endpoints were observations in the FOB, reduced motor activity, and ChEI in RBC and brain. The severity and frequency of clinical signs and ChEI were dose related and decreased with time. The lowest oral NOAEL for neurotoxicity studies with carbaryl was 1 mg/kg/day.

74.3.4 Developmental and Reproductive Toxicity

In recent studies, effects on fetal development were observed only in the presence of maternal toxicity. In a developmental toxicity study in Sprague Dawley Crl:CD (SD) BR rats, carbaryl was administered by gavage at 0, 1, 4, and 30 mg/kg/day from GD6 to 20 inclusive (Repetto-Larsay, 1998). Maternal toxicity was indicated by increased salivation and approximately 30 and 15% reductions in body weight gain and food consumption, respectively, at 30 mg/kg/day. Gestational parameters were not affected. Fetal body weights were significantly reduced at 30 mg/kg/day and associated with delayed ossification of a few bones. No increased incidence of malformations was observed at any dose level. The NOAEL for both maternal and developmental toxicity was 4 mg/kg/day.

In a developmental toxicity study in New Zealand White rabbits, carbaryl was administered by oral gavage at 0, 5, 50, and 150 mg/kg/day from GD6 to 29 inclusive (Tyl *et al.*, 1999). Maternal toxicity was indicated by reduced body weight gains (25 and 53% below control for the dosing period) at 50 and 150 mg/kg/day, respectively. Maternal food consumption during the dosing period was equivalent or slightly higher than control. Gestational parameters were not affected in any treatment group. The only evidence of fetal toxicity was a 10% reduction in weight at 150 mg/kg/day compared to control. Therefore, the NOAEL for maternal toxicity was 5 mg/kg/day and the NOAEL for developmental toxicity was 50 mg/kg/day.

In a two-generation reproduction study, Sprague Dawley Crl:CD (SD) BR rats were administered carbaryl in the diet at 0, 75, 300, and 1500 ppm (Tyl *et al.*, 2001). During premating periods, these dietary levels were equivalent, respectively, to 0, 4.67, 31.34, and 92.43 mg/kg/day for F0 males; 0, 5.56, 36.32, and 110.78 mg/kg/day for F0 females; 0, 5.79, 23.49, and 124.33 mg/kg/day for F1 males; and 0, 6.41, 26.91, and 135.54 mg/kg/day for F1 females. Parental toxicity was indicated by reduced body weight and food consumption during the premating, gestation, and lactation periods at 1500 ppm. Body weights for the F0 and Fl parental animals at 300 ppm

were consistently lower throughout the study but were generally not statistically different from controls. No effect on reproduction, fertility, or reproductive organs was observed for any treatment group. Further, for F0 and F1 adult males, no significant differences were observed for percent motile or progressively motile sperm, epididymal sperm concentration, testicular spermatid head concentration, daily sperm production (DSP), efficiency of DSP, or percent abnormal sperm.

For Fl and F2 offspring, no effects of treatment on stillbirth or live birth indices, number of live pups per litter on PND0, or sex ratio (% males) were observed. However, during the lactation period, body weights for F1 and F2 pups were significantly lower at 1500 ppm, and survival indices were decreased at 1500 ppm for F1 and F2 pups and at 300 ppm for F2 pups. The NOAEL for parental and pup toxicity was 75 ppm, and the NOAEL for reproduction and fertility was 1500 ppm.

In conclusion, studies conducted on carbaryl for regulatory purposes show no evidence of teratogenicity or reproductive toxicity.

74.3.5 Genotoxicity

In vitro gene mutation assays with carbaryl in both bacterial and mammalian cells are negative (Lawlor, 1989; Onfelt and Klasterska, 1984; Young, 1989). *In vitro* studies using Chinese hamster V79 cells indicate that carbaryl induces aneuploidy and sister chromatid exchanges (Onfelt and Klasterska, 1983, 1984). However, this cell line has been reported to contain a mutated and nonfunctional p53 protein and thus results with these cells should be interpreted with caution (Chaung *et al.*, 1997). In the presence of metabolic activation, carbaryl did induce chromosomal aberrations in an *in vitro* study with Chinese hamster ovary cells (Murli, 1989), but all *in vivo* cytogenetics studies are negative (McEnaney, 1993; Marshall, 1996). An *in vitro* DNA synthesis assay with rat primary hepatocytes is also negative (Cifone, 1991). Further, no DNA adduct formation in the liver was observed in mice administered carbaryl via the diet at 8000 ppm for 2 weeks followed by a single oral dose of C14-labeled carbaryl at 75 mg/kg (Sagelsdorff, 1994). In addition, a 6-month study in male C57BL/6 p53 heterozygous knockout mice, described in more detail in the following section, is negative (Chuzel, 2000b).

74.3.6 Carbaryl Chronic Toxicity and Carcinogenicity Studies

Two-year studies with carbaryl administered via the diet have been conducted in rats and mice. In both of these studies, the highest dose tested exceeded the maximum tolerated dose (MTD), and thus, the findings described in the following sections for these high dietary exposures cannot be considered relevant for assessment of hazard or risk.

74.3.6.1 Rat Data

Carbaryl was administered in the diet to Sprague-Dawley Crl:CD BR rats at 0, 250, 1500, and 7500 ppm (equating respectively to 0, 10.0, 60.2, and 349.5 mg/kg/day in males and 0, 12.6, 78.6, and 484.6 mg/kg/day in females) for at least 104 weeks (Hamada, 1993a). A subgroup of 10 rats/sex/group was sacrificed after 52 weeks. Although survival was comparable across all groups, 7500 ppm exceeded the MTD primarily based on significantly lower body weight gains and food consumption. Compared to controls, body weight gains for the high-dose group at week 13 were reduced by 30 to 50% and by week 105, mean body weights were 35 and 45% lower for males and females, respectively. Mean body weights also tended to be lower at 1500 ppm and by week 105 were 6 and 12% below controls for males and females, respectively. Inhibition of RBC and brain ChE was observed at 1500 and 7500 ppm at both interim and final sacrifices.

Increased incidences of microscopic findings related to treatment were observed only at 7500 ppm and consisted of transitional cell hyperplasia, papillomas, and carcinomas in the urinary bladder of males and females, pelvic epithelial hyperplasia in kidney of males with a single incidence of a kidney transitional cell carcinoma in one male, thyroid follicular cell hypertrophy in males and females with thyroid follicular adenomas in males only, and hepatocellular hypertrophy in males and females with hepatocellular adenomas in females only (Table 74.2). Increased incidences of urinary bladder transitional cell, epithelial hyperplasia in the renal pelvis, and hepatocellular hypertrophy were also observed at the 52-week interim sacrifice. Cell cycling assessments of the urinary bladder and thyroid gland from male rats and the liver from female rats of the control and high-dose groups at the 52-week sacrifice interval were performed using proliferating cellular nuclear antigen (PCNA) (Irisarri, 1996). Compared to control, a significant increase in the number of PCNA-positive urothelial cells was seen in the urinary bladder of the 7500 ppm males, while only slight increases were observed in the thyroid glands of males and the liver of females from the same treated group. The overall NOAEL for the study was 250 ppm.

74.3.6.2 Mouse Data

In a 2-year study in CD-1 mice, carbaryl was administered via dietary admixture at 0, 100, 1000, and 8000 ppm (equating to 0, 14.7, 146.0, and 1248.9 mg/kg/day in males and 0, 18.1, 180.9, and 1440.6 mg/kg/day in females) (Hamada, 1993b). Ten animals/sex/group were sacrificed after 52 weeks. Although survival was comparable across all groups, 8000 ppm exceeded the MTD based mainly on significantly lower body weight gains, clinical signs, and histopathological changes in bladder, kidneys,

TABLE 74.2 Incidence of Microscopic Findings in the Rat Carcinogenicity Study with Carbaryl (All Animals on Study)

	Males				Females			
Dietary levels in ppm	0	250	1500	7500	0	250	1500	7500
Total number of animals	70	70	70	70	70	70	70	70
Urinary bladder								
Transitional cell hyperplasia	8	7	11	51	6	4	4	56
Transitional cell papilloma	0	0	0	14	1	0	0	8
Squamous cell papilloma	0	0	0	2	0	0	0	0
Transitional cell carcinoma	0	0	0	10	0	0	0	5
Kidney								
Pelvic epithelial hyperplasia	13	10	13	29	22	39	29	21
Transitional cell carcinoma	0	0	0	1	0	0	0	0
Thyroid gland								
Follicular cell hypertrophy	2	1	1	9	3	4	2	33
Follicular cell adenoma	0	2	0	9	1	0	0	1
Follicular cell carcinoma	0	0	1	0	0	0	0	0
Liver								
Hepatocellular hypertrophy	0	1	2	38	7	6	10	34
Hepatocellular adenoma	1	1	1	1	1	0	3	7
Hepatocellular carcinoma	0	2	3	1	0	0	0	0

and spleen. Clinical signs of toxicity noted at 8000 ppm included hunched posture, thin and languid appearance, opaque eyes, urine stains, rough hair coat, soft feces, and low body temperature. Further, body weight gains were 33 and 19% lower than control for high-dose males and females, respectively, during the first 13 weeks of treatment and remained well below control gains for the entire study. Decreased RBC counts, hemoglobin concentration, and hematocrit were observed at 8000 ppm. RBC and brain ChE were decreased at 1000 and 8000 ppm at both the interim and final sacrifice intervals.

Non-neoplastic findings observed after 52 and 104 weeks of treatment included intracytoplasmic protein-like droplets in urinary bladder superficial transitional epithelium at 1000 and 8000 ppm and increased incidence of lens cataracts and increased severity of extramedullary hematopoiesis and pigment in the spleen at 8000 ppm. Also, an increased incidence of chronic progressive nephropathy was observed at the 52-week sacrifice in males at 1000 ppm and in both sexes at 8000 ppm. Cell cycling assessments of liver and kidney from male and female mice of the control and high-dose groups at the 52-week sacrifice interval were performed using PCNA (Debruyne, 1998; Irisarri, 1996). Only a slight increase in the number of PCNA-positive cells was observed in the kidney from treated males.

Vascular neoplasms involving a variety of organs, with the liver and spleen being the most often affected, were observed in all treated male groups and in females at 8000 ppm (Table 74.3). Increased incidences of tubular cell neoplasms in the kidney and hepatocellular neoplasms were also observed at 8000 ppm in males and females, respectively.

To better understand the carcinogenic potential of carbaryl, especially with regard to vascular tumors, a 6-month study was conducted with male C57BL/6 p53 heterozygous knockout mice. These mice carry only one wild-type p53 gene (p53 +/−) and are more susceptible to genotoxic carcinogens (Donehower, 1996; Tennant *et al.*, 1995). In previous work, the genotoxic compound urethane, known to produce vascular tumors in lifetime studies in mice, also induced vascular tumors in p53 heterozygous knockout mice within 6 months of administration, and D-limonene, which has been identified as a nongenotoxic carcinogen in rat only (kidney tumors due to a specific male rat mechanism), was found to be negative (Carmichael *et al.*, 2000). These results, along with the essentially zero level of vascular

TABLE 74.3 Tumor Incidence in the Mouse Carcinogenicity Study with Carbaryl (All Animals on Study)

	Males				Females			
Dietary levels in ppm	0	100	1000	8000	0	100	1000	8000
Total number of animals	80	80	80	80	80	80	80	80
	Vascular Tumors							
Total hemangiomas	1	1	2	2	2	1	1	0
Total hemangiosarcomas	1	9	11	16	4	6	4	10
Total number of vascular tumors	2	10	13	18	6	7	5	10
Vascular tumor-bearing animals	2	7	10	10	4	4	4	9
	Kidney							
Tubular cell adenoma	0	0	0	3	0	0	0	0
Tubular cell carcinoma	0	0	1	3	0	0	0	0
	Liver							
Hepatocellular adenoma	12	6	11	10	1	0	1	7
Hepatocellular carcinoma	6	7	3	6	0	1	1	2

tumors in control p53 heterozygous knockout mice, support this model and study design as being suitable to investigate low-potency compounds suspected of being vascular carcinogens.

Carbaryl was administered for 6 months to male C57BL/6 p53 heterozygous knockout mice via dietary admixture at 0, 10, 30, 100, 300, 1000, or 4000 ppm (equivalent to 0, 1.76, 5.21, 17.5, 51.6, 164.5, and 716.6 mg/kg/day, respectively) (Bigot-Lasserre *et al.*, 2003; Chuzel, 2000b). The highest dietary level was selected based on a 28-day range-finding study in which p53 wild-type C57BL/6 male mice showed a 14% loss in body weight during the first week of treatment at 8000 ppm followed thereafter by little gain and a similar but less pronounced effect at 4000 ppm (Dange, 1998). No treatment-related mortalities or clinical signs were observed in the 6-month study. A slight decrease in body weight and food consumption was noted at 4000 ppm. The only nonproliferative change observed in the study was the accumulation of globular deposits in the umbrella cell layer of the urinary bladder at 100 ppm and higher with a dose-related increase in incidence and severity. This finding was not accompanied by any sign of local irritation or hypertrophy of the bladder epithelium. Histopathological examinations revealed no evidence of carbaryl-induced neoplasms of any type; in particular, no neoplastic or preneoplastic changes were noted in the vascular tissue of any of the organs examined. The only neoplasms observed were distributed randomly within groups and were recognized as occurring spontaneously in untreated mice of this strain.

Thus, under the conditions of this study, the NOAEL of carbaryl was 4000 ppm (approximately 716 mg/kg/day) for neoplastic changes. These results were not due to the lack of sensitivity of the model, since a previous study using urethane as positive control had demonstrated the sensitivity of the model for the induction of vascular tumors. Furthermore, a good correlation has been shown between the carcinogenic responses in the p53 knockout mouse model versus the conventional mouse bioassay, suggesting that most genotoxic chemicals could be detected in the p53 knockout mouse model (Storer *et al.*, 2001). The negative results for carbaryl in p53 knockout mice along with the lack of evidence for binding to DNA or chromatid proteins show that a genotoxic mechanism is unlikely to be involved in the induction of vascular tumors in CD-1 mice by carbaryl.

74.3.7 Rat Metabolism and Toxicokinetics

As the rat is the test species for the majority of toxicity studies conducted on a pesticide, understanding the metabolism and toxicokinetics of carbaryl in this species is important for the interpretation of the toxicological profile. In addition to general metabolism studies, work has been conducted to investigate carbaryl metabolism in older rats exposed to high levels of carbaryl via the diet as well as a limited investigation of metabolism in mice. The toxicokinetics of carbaryl have also been investigated following oral, intravenous (iv), and dermal dosing. The results from all of these studies are summarized below. Earlier work on the metabolism of carbaryl has been previously summarized and reviewed (Knaak, 1971).

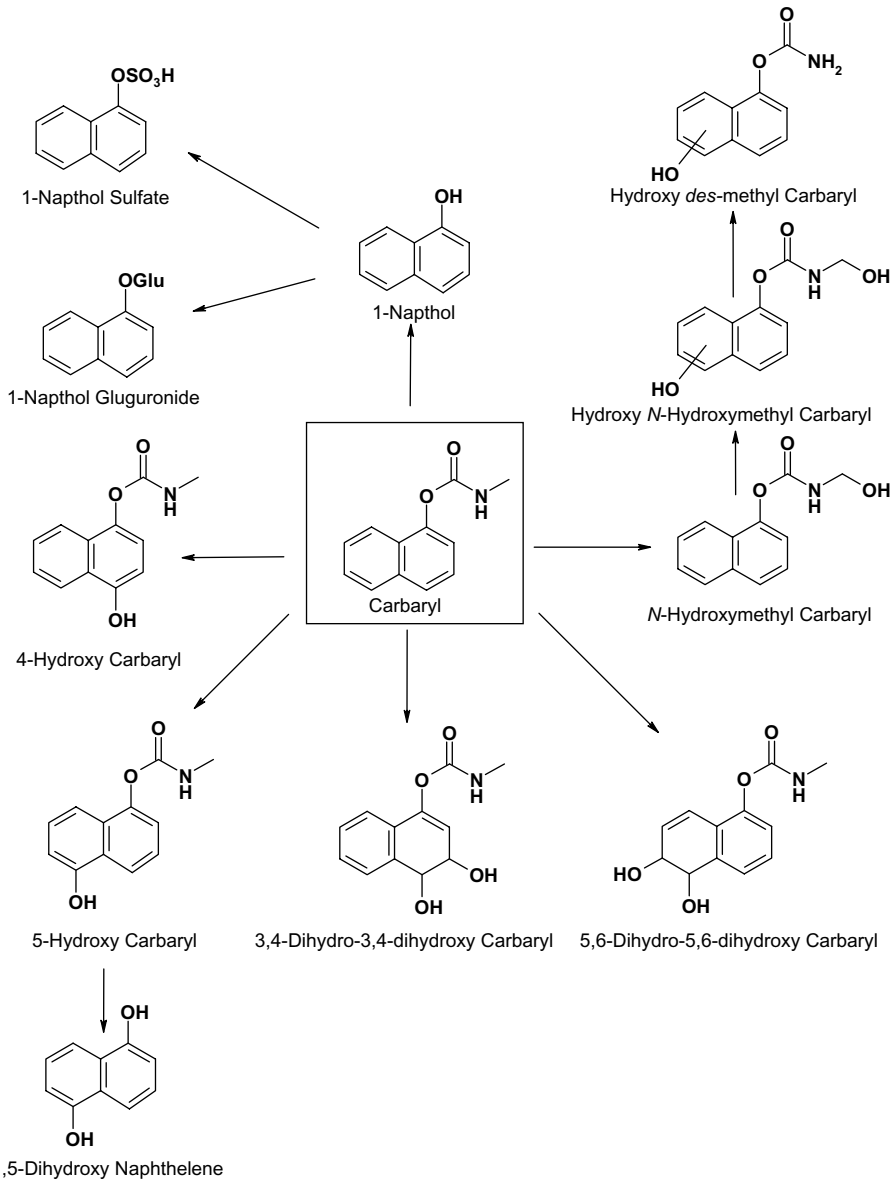

FIGURE 74.1 Proposed metabolic pathway of carbaryl in the rat.

74.3.7.1 General Metabolism Data

General metabolism work has been conducted in young Sprague-Dawley rats by Struble (1994). In these studies, a single dose of [^{14}C]carbaryl was administered by oral gavage at 1 and 50 mg/kg or intravenously at 1 mg/kg. The metabolism of carbaryl following repeated exposure was also examined in rats administered a single oral dose of 1 mg/kg [^{14}C]carbaryl following 14 consecutive days of pretreatment with nonlabeled carbaryl at 1 mg/kg. Urine and feces were collected for 168 h following dosing, at which time the animals were sacrificed for tissue collection. Metabolites were identified in urine and feces by either cochromatography with reference standards or isolation and identification by mass spectrometry. In all experiments, mass balance ranged from 96 to 104% of the administered dose, and no appreciable differences were observed in excretion rates or metabolism between the sexes.

The majority of the 1 mg/kg dose, administered either orally (single and repeated exposures) or intravenously, was excreted within 12 h of dosing, with 72 to 83% excreted in the urine and approximately 10% excreted in the feces. At 50 mg/kg, approximately 80% of the dose was excreted in urine and up to 12% in the feces, with nearly half the dose being excreted during the first 12 h postdosing and the greater part of the remainder excreted by 48 h postdosing. As the majority of the radiolabel was excreted within 24 h of dosing, very little radioactivity was detected in tissues collected at sacrifice 168 h after dosing. The main routes of metabolism were hydrolysis and oxidation, with both pathways generating hydroxylated metabolites that were then either excreted or conjugated prior to excretion. The metabolic pathway in the rat is shown in Figure 74.1. The major metabolites identified in excreta were 1-napthol

and 5-hydroxycarbaryl. A small amount of parent compound was recovered in excreta. Several metabolites, 5,6-dihydro-5,6-dihydroxy carbaryl, 3,4-dihydro-3,4-dihydroxy carbaryl, 4-hydroxycarbaryl, and 5-hydroxycarbaryl, were hypothesized as being formed via epoxide intermediates.

Carbaryl metabolism has also been investigated to determine whether a dose-related shift in the metabolic profile occurred in older rats at the dietary levels used in the 2-year rat study that might account for tumor formation (Totis, 1997). In this study, groups of male CD (Sprague-Dawley-derived) rats received carbaryl via admixture in the diet at 0, 250, 1500, or 7500 ppm for 83 days followed by oral gavage of [^{14}C]carbaryl at 2 mg/kg/day for 7 days. For purposes of comparison, a group of five male rats was administered a single oral dose of [^{14}C]carbaryl at 50 mg/kg. All animals were 15 months old at study initiation compared to the 5- to 9-week-old rats used in the study by Struble (1994).

As in the study in young rats by Struble (1994), the majority of the administered dose was excreted rapidly in the urine. For the dietary dosing regimens, urinary and fecal excretion totaled 96 to 103% of the administered dose, with most of the radioactivity being eliminated in the urine within 24 h after each administered radioactive dose. Mean levels for urinary elimination were 90, 90, and 66% of the dose administered, respectively, for the dietary groups of 250, 1500, and 7500 ppm. By comparison, following the single oral dose of 50 mg/kg, excretion in urine and feces accounted for 86 and 11% of the administered dose, respectively. Tissue levels were extremely low and represented 0.4% of the administered dose 168 h after the bolus dose of 50 mg/kg and 0.4 to 0.8% of the dose 3 days after the last administered radioactive dose in the groups initially receiving carbaryl via the diet. A shift in excretion was observed for two of the three major metabolites eliminated via the urine (Table 74.4). The glucuronide dihydro-dihydroxy 1-naphthyl-*N*-methylcarbamate metabolite, which is hypothesized to be formed by an epoxide intermediate, suggests that a higher proportion of reactive intermediates may have been present at the highest exposure level in the 2-year rat study.

A limited metabolism study has also been conducted in CD-1 male mice (10 per group) fed diets containing 0, 10, 100, 1000, or 8000 ppm carbaryl for 14 days followed by a single gavage dose of 50 mg/kg [^{14}C]carbaryl on the 15th day (Valles, 1999). Mean total radioactivity recovered, expressed as total administered dose, ranged from 88.7 to 101%, with greater amounts eliminated in the urine (55.8 to 68.9%) compared to the feces (12.2 to 18.6%). Less than 1% of the dose was found in the carcass 168 h after the radioactive dose. The four major components identified in the urine were the dihydro dihydroxy-naphthyl sulfate, the hydroxy-carbaryl glucuronide, α-naphthyl sulfate, and α-naphthyl β-D glucuronide. The first two, possibly formed by epoxide intermediates, were increased in the mice given 8000 ppm in the diet, suggesting that at high doses of carbaryl, the metabolism, distribution, and excretion pattern may be altered, with a higher proportion of reactive intermediates being formed. Comparison with results from the rat suggests that there are some differences in metabolism, although a more complete study would be required to elucidate the metabolic pathway in mice.

74.3.7.2 Toxicokinetic Data

To investigate toxicokinetics, [^{14}C]carbaryl was administered to male Sprague-Dawley rats at two dose levels via oral gavage (1.08 or 8.45 mg/kg), dermal application to shaved skin for a 10-h exposure (17.25 or 102.95 mg/kg), or iv administration (0.8 or 9.2 mg/kg) (Krolski *et al.*, 2003). Groups of four rats were sacrificed at various time points following dose administration, and total radioactive residue (TRR) levels were determined for whole blood, plasma, RBC, brain, liver, and fat. When sufficient residue was present in plasma or brain, metabolite profiles were determined and residues were characterized and/or identified.

Rapid and complete uptake, metabolic degradation, and depletion of [^{14}C]carbaryl were observed following oral and iv administration. Peak levels of radioactivity in brain, RBC, and liver were reached within approximately 15 min following oral and iv dosing, while radioactivity levels in fat peaked 30 to 60 min after dosing (Tables 74.5 and 74.6). For dermal exposure, only very low levels of radioactivity were detected in the matrices investigated. Peak levels in plasma were reached within 4 and 12 h for the low- and

TABLE 74.4 Levels of Major Urinary Metabolites Excreted by Male Rats (Approximately 18 Months Old)

Urinary metabolite[a]	0 ppm	250 ppm	1500 ppm	7500 ppm
1-Naphthyl sulfate	24.14	27.19	22.93	11.68
Glucuronide of dihydro-dihydroxy 1-naphthyl-*N*-methylcarbamate	14.52	15.65	20.55	28.46
α-naphthyl β-d glucuronide	15.69	15.51	14.03	15.15

[a]*Expressed as percentage of dose administered.*

high-dose groups, respectively, and were approximately an order of magnitude lower than peak levels detected in plasma after oral and intravenous dosing.

In all experiments, residues depleted rapidly. In plasma, parent compound was detected only within the first hour after iv administration at 9.2 mg/kg. Parent compound was also identified in brain, fat, and liver in rats receiving the oral and iv high-dose levels. In the brain, parent compound accounted for 70 to 90% of the total radioactive residue for up to 1 h after oral dosing and up to 2 h after iv dosing. By 2 h after oral and iv dosing, more than 90% of the initial level of carbaryl in the brain had been cleared. The relationship between the level of carbaryl in the brain and ChE inhibition is shown in Figure 74.2. The hydrolysis product 1-napthol was identified in all tissues analyzed, while the sulfate conjugate of 1-napthol was found only in plasma. The oxidation product *N*-hydroxymethyl carbaryl was detected in brain only within 1 h after dosing. Ring-hydroxylated carbaryl was not observed in any tissue.

The results of the oral and iv studies have been used to create a physiologically based pharmacokinetic and pharmacodynamic model (Nong *et al.*, 2008). The model describes the tissue dosimetry of carbaryl and its metabolites (1-naphthol and "other hydroxylated metabolites") as well as inhibition of ChE in RBC and brain.

TABLE 74.5 Average Total Radioactive Residue Levels (ppm) in Male Rats Following Oral Dosing

Time (h)	Oral: 8.45 mg/kg					Oral: 1.08 mg/kg		
	Plasma	RBC	Brain	Liver	Fat	Plasma	RBC	Brain
0.25	7.19	2.56	1.97	20.95	3.58	1.44	0.44	0.13
0.5	7.71	2.59	1.15	13.45	3.38	1.19	0.32	0.06
1	7.32	2.24	0.62	8.12	5.33	0.81	0.18	0.03
2	3.45	0.61	0.21	2.57	1.27	0.54	0.10	0.03
4	1.59	0.36	0.11	1.64	0.31	0.40	0.11	0.02
6	1.14	0.41	0.10	1.90	0.24	0.19	0.07	0.01
12	0.53	0.15	0.06	0.69	0.10	0.06	0.02	0.01
24	0.06	0.04	0.01	0.14	0.02	0.01	0.01	0.00

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TABLE 74.6 Average Total Radioactive Residue Levels (ppm) in Male Rats Following Intravenous Dosing

Time (h)	Intravenous: 9.2 mg/kg					Intravenous: 0.8 mg/kg		
	Plasma	RBC	Brain	Liver	Fat	Plasma	RBC	Brain
0.083	11.74	10.18	13.22	24.67	12.07	2.13	1.06	0.74
0.167	12.36	9.09	10.71	27.72	15.61	1.83	0.91	0.41
0.333	14.32	7.34	7.86	25.53	21.18	1.69	0.67	0.23
0.5	12.29	5.51	6.70	19.50	28.46	1.43	0.49	0.14
1	11.30	3.23	2.45	13.05	16.18	0.85	0.30	0.06
2	7.78	2.53	1.09	7.17	8.48	0.58	0.15	0.03
4	3.50	1.04	0.29	2.70	1.32	0.21	0.10	0.01
8	1.50	0.65	0.15	1.59	0.21	0.11	0.05	0.01

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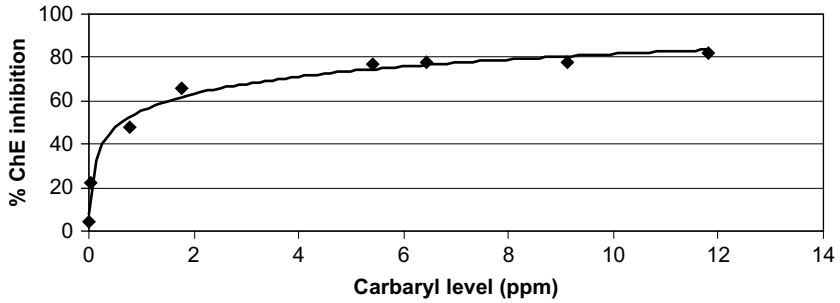

FIGURE 74.2 Brain ChEI as a function of carbaryl level in the brain of male rats following IV dose of 9.2 mg/kg.

CONCLUSION

Carbaryl belongs to the *N*-methyl carbamate chemical family. Carbaryl is moderately toxic by the oral route and exhibits a low level of toxicity following dermal or inhalation exposures. As with most carbamates, the cholinergic signs and symptoms associated with acute poisoning by carbaryl appear rapidly. In animal studies following single and repeated dosing, the carbamylation of ChE is reversible, and at oral exposures of 50 mg/kg or less, enzyme activity is similar to baseline values within 24 h after exposure. This finding is consistent with metabolism and toxicokinetic studies showing that the majority of a dose is rapidly metabolized and excreted within 24 h after either single or repeated administration. In neurotoxicity testing, the most sensitive endpoints were observations in the FOB, reduced motor activity, and ChEI in RBC and brain. The severity and frequency of clinical signs and ChEI were dose related and decreased with time.

In genetic toxicity testing, some evidence of chromosomal aberrations has been observed in *in vitro* studies with carbaryl. However, all *in vivo* studies, including a 6-month feeding study in the p53 knockout mouse model, are clearly negative. In long-term feeding studies, neoplastic findings were observed in both rats and mice. In general, these findings were observed only at dose levels exceeding the MTD. However in male mice, the incidence of vascular tumors was elevated at all exposure levels. When tested in the p53 knockout mouse model, no evidence of carbaryl-induced neoplasms of any type, in particular, no neoplastic or preneoplastic changes, were noted in the vascular tissue of any of the organs examined. These results for carbaryl in p53 mice along with the lack of evidence for binding to DNA or chromatid proteins show that a genotoxic mechanism is unlikely to be involved in the induction of vascular tumors by carbaryl.

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