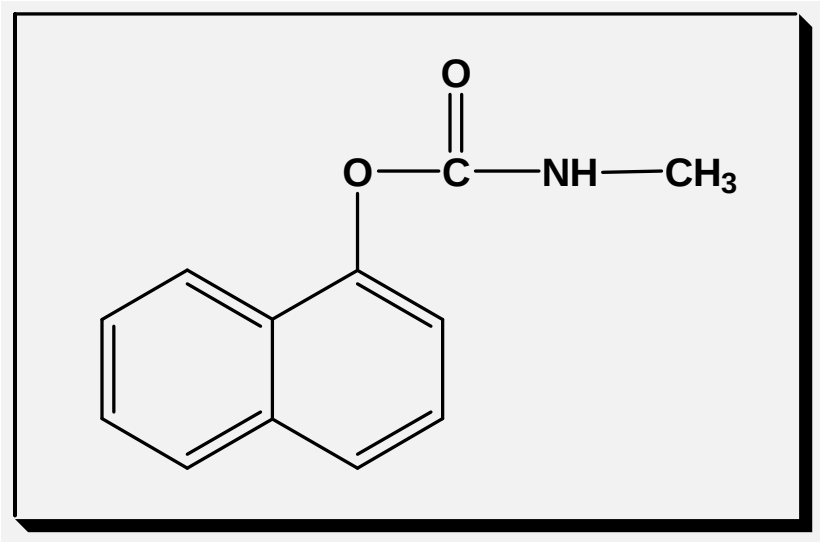
Carbaryl
(1-naphthyl methylcarbamate)

DIETARY RISK CHARACTERIZATION DOCUMENT



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I. SUMMARY

Carbaryl (1-naphthyl N-methylcarbamate; MW, 201.22) is a broad spectrum insecticide, with additional registered uses as a molluscicide. It is effective both after ingestion by the targeted pest or after absorption following direct bodily contact. As a member of the carbamate class of pesticides, carbaryl action is based on its ability to inhibit acetylcholinesterase (AChE) in the central and peripheral nervous systems of the target species. Its toxicity in mammalian systems is also based on this property, though the involvement of other toxic mechanisms is not ruled out.

Plant systems upon which carbaryl is used include citrus and other fruits, vegetables, forage, forests, field crops, nuts, ornamentals, rangeland, turf and shade trees. In addition, there are significant residential lawn, garden and pet uses. Carbaryl formulations include aqueous dispersions, baits, dusts, emulsifiable concentrates, flowables, granules, soluble concentrates, suspension concentrates, wettable powders, water dispersible granules and impregnated materials.

Originally manufactured by Union Carbide and now by Bayer Crop Science, carbaryl was first registered for use on cotton in the United States in 1959. More than 60 federal food tolerances are now in effect for this chemical. As of October 2009 there were 47 formulations containing carbaryl registered in California, with concentrations ranging between 0.126% and 99%. Several of these formulations also contain metaldehyde.

Illness and injury reports

Carbaryl alone. From 1992-2007 there were 38 illness reports classified as definitely, probably, or possibly associated with the exposure to carbaryl in California, an average of about 2 illnesses per year. Among the symptoms reported were systemic illnesses, (including respiratory illnesses), skin injuries, and eye injuries. Individuals involved in the handling process (applicators, mixer/loaders, flaggers) reported 11 illnesses and injuries. Sixteen of the 38 illnesses/injuries resulted from agricultural carbaryl applications. There were 14 days of disability and no hospitalizations resulting from agricultural carbaryl use. For non-agricultural use scenarios there were 30 days of disability (of which 18 days are suicide-associated) and 26 days of hospitalization (23 days are suicide-associated).

Carbaryl in combination. From 1992-2007, there were 60 illnesses classified as definitely, probably, or possibly associated with exposure to carbaryl used in combination with other pesticides in California. For the 16-year period, this averages to 4 illnesses per year. The larger number of illnesses when carbaryl is in combination with other pesticides may be a reflection of the toxicity of the other pesticides in the mixture, or it may be that carbaryl is used more often with other pesticides than alone. Forty-eight of the 60 cases resulted from agricultural activities. Among the 48 cases, 16 illnesses and injuries occurred during the handling process (applicator, mixer/loader, flagger). There were 52 days of disability and no days of hospitalization resulting from agricultural uses. For non-agricultural uses there were 6 days of disability and no hospitalization reported.

Environmental fate

Despite a low vapor pressure and low Henry's Law constant, carbaryl has been detected in the air, both at application sites and remotely to such sites. Association with water droplets or particulates may play a role in this process. Hydrolysis, which is favored at pH 7 and above, and

photolysis both occur under aqueous conditions. The presence of microorganisms is expected to enhance hydrolytic degradation. 1-naphthol, methylamine and CO_2 were identified as hydrolytic breakdown products. The major photolysis product is 1-naphthol, which photooxidizes to 2-hydroxy-1,4-naphtho-quinone under basic conditions. Though relatively insoluble in water, carbaryl has been detected in both surface water and groundwater. Hydrolysis, photolysis and microorganism-mediated degradation also occur in soil, where, interestingly, breakdown is enhanced under *acidic* conditions. Carbaryl has a moderate ability to bind to soil, a process which is also favored under acidic conditions. Mud has been shown to enhance the removal of carbaryl from aqueous systems, presumably by preferential binding. Carbaryl tends to be associated with the top 0 - 0.15 meters of soil, showing a dissipation half-life of 0.76 - 10.9 days. Exchangeable cations such as potassium enhance the adsorption of carbaryl to soils, a process that is also enhanced by the presence of organic matter. Carbaryl and its major metabolite, 1-naphthol, are toxic to some beneficial soil-dwelling microorganisms, despite the fact that some bacterial species and at least one fungus can metabolize carbaryl. Microbial degradation of carbaryl is enhanced under anaerobic conditions. Carbaryl is toxic to fish, aquatic invertebrates and non-target insects (*eg.*, honeybees), but relatively non-toxic to birds. Metabolism of carbaryl in plants is similar to that in animal systems (see below).

Pharmacokinetics

Orally administered carbaryl is excreted primarily through the urine in rats during the first 24 hours, though substantial residues appear in feces and in exhaled air as CO_2 (detectable when carbaryl is labeled in the carbonyl or N-methyl carbon, but not when labeled in the naphthalene ring). Metabolites were conjugated with sulfate or glucuronic acid. For animals receiving 1 mg/kg, about half the dose was detected in the urine during the first 6 hr, with 80-90% by 24 hr and only slightly more by 168 hr. For animals receiving 50 mg/kg, urinary excretion was slower: 12-20% by 6 hr, 64%-69% by 24 hr, and 78-81% by 168 hr. Fecal excretion was also significant, though it comprised a lesser proportion of the administered dose than urinary excretion; by 168 hr, 6%-13% of the dose appeared in the feces. A separate study in rats examined the appearance and disappearance of [naphthyl-1-^{14}C]-carbaryl in the blood and other tissues after exposure by the oral (1.08 or 8.45 mg/kg), dermal (17.25 or 102.95 mg/kg) and intravenous (0.80 or 9.20 mg/kg) routes. Peak levels of radioactivity were detected in the blood at 15 and 30 min for the low and high dose oral treatments, respectively; at 4 and 12 hr for the dermal applications; and were already maximal by the first time point (5 min) for the iv injections. *Oral dosing*: by 24 hr, radioactivity levels had decreased to 0.81%-2.4% of peak levels in blood fractions (both doses), 0.60%-2.4% in brain (both doses), 0.67% in liver (high dose only) and 0.32% in fat (high dose only). *Dermal dosing*: by 24 hr, radioactivity levels had decreased to 15.9%-25.8% of peak levels in blood fractions (both doses), 27.1%-30.6% in brain (both doses), 24.4% in liver (high dose only) and 15.6% in fat (high dose only). *Iv dosing*: by 24 hr, radioactivity levels had decreased to 4.6%-10.5% in blood fractions (both doses), 1.1%-1.3% in brain (both doses), 5.7% in liver (high dose only) and 0.72% in fat (high dose only).

The recovery kinetics in other laboratory species examined (guinea pig, sheep) appeared generally similar to those in the rat, though there were problems with the latter studies, which were conducted during the 1960s, tested few animals and left large fractions of the administered dose unanalyzed. A possible exception to the rat model was the dog, where approximately equal fractions were excreted in the 24-hr urine and feces, though these data, also collected in the 1960s by the same lab, had similar problems. Speculation about the tendency toward tumor formation at high doses in the more recent mouse study centered on a shift in the urinary metabolite pattern at 8000 ppm, with increases in compounds derived from

epoxide intermediates.

Three major metabolic pathways, presumably predominantly hepatic, were identified in the rat: (1) arene oxide-mediated hydroxylation and subsequent conjugation, (2) hydrolytic decarbamylation to form 1-naphthol and subsequent conjugation, and (3) oxidation of the N-methyl moiety. Three urinary metabolites found in rat urine - 1-naphthyl glucuronide, 1-naphthyl sulfate and 4-(methyl-carbamoyloxy)-1-naphthyl glucuronide - were not found in dog urine. In addition, very few hydrolytic products were found in the urine of a single dosed monkey. The toxicologic significance of these apparent metabolic species differences was not clear. Humans do appear to have the ability to decarbamylate carbaryl since carbaryl factory workers were shown to excrete 1-naphthyl glucuronide and 1-naphthyl sulfate, leading to speculation that humans are more similar to rats in their pharmacokinetic handling of carbaryl. However, another study showed that intentionally dosed humans excreted only 25-30% of the carbaryl in the urine at 24 hours, suggesting that the fate of a significant fraction of the dose was unknown.

Hazard identification

Acute oral toxicity. The acute toxicity of carbaryl results from its ability to carbamylate, thus inhibit, acetyl cholinesterase (AChE) at synapses and neuromuscular junctions. Consequent local accumulations of acetylcholine (ACh) generate cholinergic effects. Due to the reversibility of the carbamate-AChE bond, recovery is expected when exposures are low.

Rats exhibited acute oral LD_{50}s between 233 and 840 mg/kg, while mice were between 108 and 650 mg/kg. Two critical acute oral regulatory values were established in this assessment representing endpoints of stronger (#1 below) or weaker (#2 below) experimental support. (1) A critical acute NOEL of **1 mg/kg** was based on the appearance both of clear, statistically significant cholinergic signs (slight tremors, slight hypotonic gait, slight ataxic gait and pinpoint pupils) detected in FOB testing and of statistically significant body weight gain decrements in pregnant rats at a gavage dose 10 mg/kg. Despite daily dosing between gestation day 6 and *post partum* day 10, the cholinergic signs were first noted on day 6, qualifying them as acute responses. The decrease in body weight gain was also acute or near-acute, as the deficit was apparent by the first weight measurement on gestation day 9, three days after the commencement of dosing on day 6. Statistically lowered RBC and brain cholinesterase activities were also measured at 10 mg/kg, though it is not known if these effects were acute or required several daily exposures. However, it is worth noting that the RBC ChE was suppressed by almost 20% on gestation day 6. (2) A critical acute LED_{10} of **0.25 mg/kg** was based on benchmark dose modeling of the incidence of slight hypotonic gait in pregnant rats in the same gavage study over the study dose range of 0.1 - 10 mg/kg. While this was a developmental neurotoxicity study in which the dams were exposed between gestation day 6 and *post partum* day 10 inclusive, the acute nature of this sign was highly probable.

Three acute toxicity studies conducted in a single laboratory established low dose acute LOELs at 10 mg/kg in the rat, similar to the level at which there were overt clinical signs, body weight gain deficits and suppressed cholinesterase activities in the critical study, thus supporting its observations. In addition, USEPA established an LED_{10} of 1.1 mg/kg based on inhibition of brain cholinesterase activity in postnatal day 11 rats following a single gavage exposure to carbaryl.

Chronic oral toxicity. The critical chronic oral LOEL was based on 14% inhibition of brain cholinesterase activity at the low dose of 3.4 mg/kg/day ($p>0.05$) in males at 52 weeks in the 1-yr dog dietary study (20% in females at 3.7 mg/kg/day, $p<0.05$). RBC cholinesterase showed

statistically significant inhibition at the mid and high doses (11.0-♂ / 11.2-♀ mg/kg/day and 33.8-♂ / 34.4-♀ mg/kg/day, respectively) at all treatment intervals (weeks 5, 13, 26 and 52), while non-statistically significant inhibition was detected at the low dose (up to 14% in males at week 13 and 13% in females at week 5, the first measurement). Plasma cholinesterase activites were statistically suppressed in females at all doses for weeks 5, 13 and 26 (up to 23% at the low dose). Statistical significance in males occurred at the mid and high doses only.

The benchmark dose approach was employed to estimate a regulatory chronic LED_{10} value. The Hill algorithm for continuous data generated the most appropriate curve to fit the female Week 52 brain cholinesterase data. The 10% benchmark response rate was chosen in recognition of the fact that neither overt clinical signs nor histopathology were observed throughout the study, even at the high dose of ~34 mg/kg/day. The critical chronic LED_{10} for brain cholinesterase inhibition in females using the Hill algorithm was **0.5 mg/kg/day** (ED_{10} = 1.7 mg/kg/day). This value was used to evaluate the non-oncogenic risks from annual (*i.e.*, chronic) exposure to carbaryl.

Genotoxicity. Carbaryl tested positive in one of five gene mutation studies, four of six chromosomal aberration studies and two of four DNA damage studies reviewed for this assessment. It should thus be viewed as potentially genotoxic. Virtually all of the positive studies were performed *in vitro*, which made them less relevant than *in vivo* studies to the whole organism. One study in V79 Chinese hamster fibroblasts showed that, like carbaryl, the carbaryl metabolite ∝-naphthol was toxic and induced c-mitosis, an aberrant form of mitosis that may reflect effects on mitotic spindle formation. Nitrosocarbaryl, which caused single strand breaks in cultured human fibroblasts, was more efficiently formed in the guinea pig stomach than in the rat stomach, an effect attributed to the more acidic conditions of the guinea pig stomach.

Oncogenicity. Carbaryl administered through the diet to mice over a two-year period induced hemangiosarcomas and hemangiomas in both sexes, hepatocellular carcinomas and adenomas in females, and kidney tubular adenomas and carcinomas in males. Similar treatment in rats led to hyperplastic and neoplastic signs in the urinary bladder of both sexes. These included hyperplasia, transitional cell papillomas, transitional cell carcinomas, squamous metaplasia, high mitotic index and atypia. Tumors did not appear within a 6-month time frame in p53 knockout mice, suggesting that carbaryl does not act through a p53-dependent mechanism. However, in view of the positive genotoxicity tests (see previous section), genotoxicity could not be excluded as a possible component in carbaryl-induced cancers in mice. For this reason, and for reasons of appropriate curve-fitting, the quantal linear model was used to define carbaryl's tumor potency with respect to hemangiosarcomas / hemangiomas in male mice.The potency value in "at risk" animals, defined as the slope of the dose-risk relation, was $1.45x10^{-3}$ $(mg/kg/day)^{-1}$ at the 95% upper bound and $7.2x10^{-4}$ $(mg/kg/day)^{-1}$ at the maximum likelihood estimate using the quantal linear algorithm after omission of the top dose. These potency values were extrapolated to humans using a dose equivalence based on body weight raised to the 3/4 power or, in this case, a factor of 0.153. Thus the human equivalent potency values were **$1.01x10^{-2}$ $mg/kg/day^{-1}$** (the 95% upper bound estimate) and $5.03x10^{-3}$ $mg/kg/day^{-1}$ (the maximum likelihood estimate).

Reproductive toxicity. Several studies, both epidemiologic and animal, suggested that carbaryl is toxic to the reproductive systems of both males and females. An epidemiologic study of agricultural workers indicated that the relative risk for miscarriage approximately doubled when carbaryl usage by males was combined with one of two other exposure categories,

including “crop herbicide application” and “application of crop insecticides and fungicides”. An earlier epidemiologic study failed to establish “a definitive link between carbaryl exposure and human seminal defects”, though the data were suggestive of an increase in oligospermia (sperm count <20x10^6/ml) and teratospermia (>60% abnormal sperm forms) among workers and ex-workers in a carbaryl production facility. A more recent study showed sperm toxicity in occupationally exposed factory workers. In addition, a positive correspondence between urinary 1-naphthol levels and various indicators of sperm toxicity was demonstrated among males seeking diagnoses for infertility.

Laboratory animal studies were equivocal in this regard. The clearest positive results came from gavage studies in rats demonstrating impacts on testicular enzymes, sperm counts, sperm motility, sperm morphology and testicular morphology at a daily gavage dose of 50 mg/kg/day (5 days/week, 90 days). An older study in gerbils also demonstrated impairment in several reproductive indices.

Developmental toxicity. Outside of possible developmental delays likely mediated by maternal weight gain suppressions, there was minimal evidence from guideline studies for carbaryl-mediated developmental toxicity in rats and rabbits (though omphalocele was present at relatively high doses in an older rabbit gavage study from the open literature). However, a 1960s-era oral study in dogs demonstrated severe maternal and fetal effects following gestational exposure: (1) increased dystocia at all dose levels (3.125-50 mg/kg/day); (2) three mothers sustaining total fetal deaths (one each at 6.25, 25 and 50 mg/kg/day); (3) decreased pup weight gains in the combined treatment groups; (4) decreased conception rate at the high dose; (5) no pups born alive at the high dose; (6) decreased percentage of pups weaned, an effect possibly present at as low as 6.25 mg/kg/day; and (7) increased litters with pups bearing abnormalities at and above 6.25 mg/kg/day. The abnormalities included “abdominal-thoracic fissures with varying degrees of intestinal agenesis and displacement, varying degrees of brachygnathia, ecaudate pups [*i.e.*, without a tail], failure of skeletal formation, failure of liver development, and superfluous phalanges”.

Toxicity of 1-naphthol. Human exposure to 1-naphthol likely occurs through the metabolism of carbaryl or naphthalene. Exposure is also plausible through (1) the use of this chemical in microscopy, (2) as a coupler in cosmetic hair dyes, or (3) in the manufacture of dyes and intermediates. *Pharmacokinetics.* Male mice receiving 1-naphthol by oral gavage showed a 24-hr elimination of 68% in the urine and 13% in the feces; the major metabolites were 1-naphthyl glucuronide and 1-naphthyl sulfate. A very limited study using three human male volunteers determined that 1-naphthol contained in an ointment was rapidly absorbed. *Acute toxicity.* The rat LD_{50} fell between 1870 and 2590 mg/kg. Signs and symptoms after acute exposure in rats included tremors, abnormal respiration, subdued behavior, piloerection and labored breathing. Histopathologic changes were noted in the kidney and gut. *Subchronic toxicity.* Subchronic oral exposure resulted in gut erosion at a high dose of 200 mg/kg/day and a LOEL of 50 mg/kg/day based on weight gain decrements and possible effects on female white blood cell counts. Hematologic analysis revealed an apparent dose-related rise in white blood cell counts among females, though the report claims that these increases were within the historical control range for the laboratory. Body weight gains were suppressed at all doses. *Irritation.* 1-Naphthol was irritating to skin and eyes of rabbits. *Teratogenicity / embryotoxicity*. There was no evidence of any teratogenic or other adverse effect in the developing embryo / fetus after dermal exposure up to 10 mg/kg/day every 3 days throughout gestation in the rat. *Mutagenicity / genotoxicity*. Nine *Salmonella* / Ames studies using various strains were negative. One study

was positive in strain TA1538, with a maximal effect at 500 mg/plate in the presence of S9 microsomes (negative in three other strains). Another study was positive in five strains in the absense of S9 microsomes. A Rec assay in *B. subtilis*, was positive in the absence of S9 and negative in the presence of S9. A plethora of other genotoxicity study types were negative. However, 1-naphthol, like carbaryl, induced an aberrant form of mitosis called c-mitosis that may reflect effects on mitotic spindle formation in V79 cells.

Toxicity of methylamine. Methylamine is produced upon hydrolytic breakdown of carbaryl, which occurs under alkaline conditions. This compound is known for its irritant effects in eyes, nose and throat upon brief exposures to 20 - 100 ppm. Severe methylamine exposure may lead to pulmonary edema. The oral LD_{50} in rats was 100 - 200 mg/kg. The L5178Y mutagenicity assay was positive. Gavage exposure of mice to 122 mg/kg methylamine for 5 days produced a statistically significant 27% drop in white blood cell counts.

Dietary exposure, risk calculations and risk appraisal

The potential for non-oncogenic health effects resulting from exposure to carbaryl was expressed as the Margin of Exposure (MOE). The MOE is defined as the ratio of the critical LED_{10} value divided by the estimated exposure. A MOE of >100 was considered to be protective of human health when the relevant adverse effects were observed in animal studies, as is the case in the present assessment.

Dietary pesticide exposure is the product of the amount of food that is consumed and the concentration of the pesticide residue in that food. DPR dietary assessments consider only those commodities that carry a tolerance for the pesticide in question. For carbaryl, this included several dozen commodities, in addition to drinking water. Carbaryl residues in food were established mainly from the USDA's Pesticide Data Program, with a small minority of residue values coming from FDA databases and field trials. The levels of commodity consumption came from the USDA's 1994-1998 Continuing Survey of Food Intake by Individuals (CSFII). A dietary exposure program developed by Exponent / Novigen Science, the Dietary Exposure and Evaluation Module (DEEM-FCID®), was used to calculate exposures for the US population as a whole and 18 separate geographic, ethnic and age-based subpopulations.

For estimating acute exposure, either the highest residue values below the tolerance or the distribution of residues were considered. Exposure to carbaryl's most prominent degradate, 1-naphthol, was not included in the analysis. While the carbaryl tolerances for most commodities included 1-naphthol, and while the PDP program included residue data for this compound, it exhibited a substantially higher acute LD_{50} and subchronic LOEL than carbaryl. In addition, as a decarbamylated degradate, 1-naphthol was unlikely to be an effective cholinesterase inhibitor, which was the basis for the acute and chronic critical carbaryl NOELs.

Acute exposure to carbaryl was calculated on a per-user day basis, which included only the days of survey in which each separate commodity was consumed. A tiered approach was used to estimate acute dietary exposure. Residue values were set at tolerance (Tier 1), the highest measured value or limit of detection for each commodity (Tier 2), or were the result of a combination of the distributions of consumption and measured residue values (Tier 3 / Monte Carlo). Only data from Tiers 2 and 3 are presented in this assessment, as Tier 1 was viewed as a means to trigger the higher tiers.

For Tier 2, the highest exposures were predicted for infants and young children (1-2 yr and 3-5

yr age groups in particular). Acute Critical Exposure Commodity (CEC) analysis under Tier 2 identified blueberries, peaches, apricots, apples, asparagus, olives, grapes and strawberries as making substantial (i.e., >5%) contributions to the acute dietary exposure of the highest exposure groups (infants, children 1-2 yr and children 3-5 yr) under Tier 2. The high contributing commodities in the US population as a whole included apples, asparagus and blueberries.

Tier 3 refinement of the acute dietary exposure assessment using distributionally-determined residue values was made necessary by the sub-100 MOEs obtained under Tier 2 (see following paragraphs). Lower exposure predictions and consequently higher MOEs were the result. Critical Exposure Commodity (CEC) analysis under Tier 3 identified cherries, rice, blueberries, peaches, pineapples, and plums (infants and children 1-2 yr) as major contributors. Cherries and plums were the major contributors for the US population as a whole.

Acute MOEs calculated under Tiers 2 and 3 were as follows:

♦ Acute toxicity (NOEL = 1 mg/kg): Margins of exposure (MOEs) based on the highest residue determination for each commodity (Tier 2) were below the health protective standard of 100 for several subpopulations at the 95th user day percentile. At the 99th user day percentile, most populations showed MOEs less than 100, reaching as low as 24 for all infants and non-nursing infants <1 yr. A refined Tier 3 / Monte Carlo analysis was triggered because of these low values.

There were no subpopulations exhibiting MOEs below 100 at the 95 or 99th user day percentiles using Monte Carlo analysis (Tier 3). However, at the 99.9th percentile, three subpopulations exhibited MOEs below 100: all infants (MOE=91), non-nursing infants <1 yr (MOE=76) and children 1-2 (MOE=92).

♦ Acute toxicity (LED_{10} = 0.25 mg/kg): Margins of exposure (MOEs) based on the highest residue determination for each commodity (Tier 2) were below the health protective standard of 100 for all subpopulations at the 95th and 99th user day percentiles. The lowest MOEs at any percentile were found among infants, reaching 6 at the 99th percentile and 16 at the 95^{th} percentile for non-nursing infants <1 yr. For all infants these values were 6 and 19, respectively, while for nursing infants <1 yr they were 9 and 17. Because these values were so low, Tier 3 / Monte Carlo analysis was triggered.

Monte Carlo-derived MOEs at the 99.9th percentile fell below the health-protective standard of 100 for almost all subpopulations. As with the point estimate data, the lowest MOEs occurred among infants and young children 1-2 years, reaching 19 for non-nursing infants <1 yr at the 99.9th percentile. Even at the 99^{th} percentile there were many subpopulations registering MOEs below 100, including one (non-hispanic non-white non-black) that included adults. All subpopulations showed MOEs greater than 100 at the 95^{th} percentile in the Monte Carlo analysis.

A tiered approach was not taken in the calculation of chronic exposure and MOEs, which instead were determined using per-capita mean consumption estimates. Children 1-2 yr exhibited the lowest MOE (highest chronic exposures) at 763, followed by non-nursing infants with an MOE of 827. These were not considered to constituted a chronic dietary health concern.

Oncogenic risk (or “lifetime risk”) was expressed as the product of the projected exposure multiplied by the 95% upper bound on potency. The resultant unitless value represents the total extra cases expected as a result of "lifetime" exposure to carbaryl. In the context of the DEEM-FCID®-based dietary assessment, the amortized lifetime exposure was the same as the chronic exposure output for the various adult subpopulations examined. Oncogenic risk ranged between $2.54x10^{-6}$ and $3.83x10^{-6}$ for these subpopulations. For the US population as a whole, the oncogenic risk was $2.90x10^{-6}$. As these values were higher than the negligible risk standard of 10^{-6}, carbaryl was considered to constitute an oncogenic risk by the dietary route under current use conditions.

Reference doses (RfDs)

Oral doses of a pesticide below a calculated RfD were unlikely to pose a risk to human health. RfDs were calculated for acute and chronic dietary exposure to carbaryl by dividing the critical oral NOEL or LED_{10} by an uncertainty factor of 100 to account for variations in sensitivity in animal and human populations.

Using the critical oral NOEL of 1 mg/kg, the resultant RfD_{acute} was **0.01 mg/kg**. Acute dietary exposures calculated using the point estimate approach at the 95th percentile exceeded the RfD_{acute} for all infants, nursing infants <1 yr, non-nursing infants <1 yr, children 1-2 yr and children 3-5 yr. Using the Monte Carlo approach at the 99.9th percentile, the RfD_{acute} was exceeded for all infants, non-nursing infants <1 yr and children 1-2 yr. These data suggest that mitigation of carbaryl in food sources may be warranted.

Using the critical LED_{10} of 0.025 mg/kg, the resultant RfD_{acute} was **0.0025 mg/kg**. Acute dietary exposures calculated using both the point estimate approach at the 95th percentile and the Monte Carlo approach at the 99.9th percentile exceeded the RfD_{acute} for all of the subpopulations that yielded sufficient user day data. These data also suggest that mitigation of carbaryl in food sources may be warranted.

Using the chronic LED_{10} of 0.5 mg/kg/day, the resultant $RfD_{chronic}$ was **0.005 mg/kg/day**. The highest chronic dietary exposure estimate, which was recorded for children 1-2 yr, was only 13% of the $RFD_{chronic.}$ By this measure, chronic exposure to carbaryl does not pose a non-oncogenic risk to any sub-population and does not currently warrant mitigation.

Tolerance assessment

A separate acute tolerance assessment was conducted for each “high-contributor” commodity, defined for the tolerance assessment as those commodities providing greater than 5% of the total carbaryl consumption in the Tier 2 point estimate calculations at the 95th percentile as indicated by the Critical Exposure Contribution analysis for infants and children 1-2 yr. These commodities included blueberries, peaches, asparagus, apricots, blackberries and apples. MOEs of less than 100 were indicated for every commodity examined where there were sufficient user days available, regardless of which critical acute toxicity value was used in the DEEM calculation. Consequently, tolerance values may require reevaluation in many cases.

A chronic exposure assessment using residue levels set to the established carbaryl tolerances was not attempted, as it was highly improbable that single or multiple commodities containing pesticide levels at tolerance would be consumed habitually.

Comparison with USEPA's 2007 Reregistration Eligibility Document for carbaryl

Acute assessment: USEPA's acute "point of departure" (PoD) was 1.1 mg/kg, a benchmark dose-derived LED_{10} value from brain cholinesterase inhibition data in postnatal day 11 rats. The USEPA PoD was essentially equivalent to DPR's critical acute NOEL of 1 mg/kg, but ~4-fold greater than DPR's critical acute LED_{10} of 0.25 mg/kg. Combined with DPR's more selective use of surrogate residue data files in the Tier 3 / Monte Carlo exposure analysis, these critical toxicity values largely account for the differences between the two agencies in their estimation of risk. (DPR also used, wherever possible, only residue data collected in California.) USEPA concluded that dietary exposure to carbaryl was lower than their target value of 100% of the "acute population adjusted dose" (aPAD) at the 99.9th user day percentile for all subpopulations analyzed. For example, the aPAD was 29% for the US population (equivalent to a MOE of 345), 40% for all infants (equivalent to a MOE of 250) and 60% for children 1-2 yr (equivalent to a MOE of 167). Equivalent Tier 3 MOEs calculated by DPR for those subpopulations at the 99.9th percentile were 164, 92 and 92 (NOEL = 1 mg/kg) and 41, 23 and 23 (LED_{10} = 0.25 mg/kg). DPR thus anticipates a higher level of risk from dietary exposure to carbaryl, regardless of the critical acute toxicity term used to calculate MOEs.

Chronic assessment: USEPA did not estimate a chronic PoD for carbaryl, as it did not consider that carbaryl, with its rapid dissociation from the cholinesterase enzyme, posed a chronic risk. DPR's chronic oral LED_{10} was 0.5 mg/kg/day (ED_{10} = 1.7 mg/kg/day). The lowest chronic MOE calculated by DPR, 763 for children 1-2 yr, was considered to represent a negligible risk due to chronic dietary exposure.

Oncogenicity: USEPA regards carbaryl as a "likely human carcinogen". USEPA and DPR agreed that the formation of hemangiosarcomas in male mice, observed in the 2-year study was the most sensitive oncogenic endpoint (though DPR included hemangiomas). The 95% upper bound human equivalent potency slope values calculated by the two agencies were not similar, however, differing by a factor of 11.5-fold (USEPA: 8.75×10^{-4} mg/kg/day^{-1}; DPR: 1.01×10^{-2} mg/kg/day^{-1}). The major source of this discrepancy was almost certainly DPR's choice to eliminate the high dose in conducting the potency analysis. USEPA's consequent risk value, 2.8×10^{-8} for the US population did not exceed the negligible risk standard of 10^{-6}. DPR's range of risk values, 2.54×10^{-6} - 3.83×10^{-6} for the adult subpopulations analyzed and 2.9×10^{-6} for the US population in general, clearly did exceed that standard.

Reproductive and developmental toxicity: USEPA did not express a high level of concern about

the potential for reproductive or developmental toxicity, as the NOELs in the contract studies were higher than the critical acute PoD of 1.1 mg/kg. Application of a Food Quality Protection Act safety factor of 1 reflected this view. DPR viewed reproductive toxicity mainly through the lens of epidemiologic studies, which indicated potential reproductive problems in males. DPR also considered the developmental toxicity evident in an open literature study in beagle dogs to reflect a potential developmental risk.

California Proposition 65 (The Safe Drinking Water and Toxic Enforcement Act of 1986)

Carbaryl has been listed as a developmental toxin in males under Proposition 65 since August 7, 2009. In addition, the Office of Environmental Health Hazard Assessment announced in November 2009 its intent to list carbaryl as a carcinogen under Proposition 65.

II. INTRODUCTION

A. CHEMICAL IDENTIFICATION

Carbaryl (1-naphthyl N-methylcarbamate; MW, 201.22) is a broad spectrum insecticide used to control over 100 insect species, including moths, beetles, cockroaches, mosquitos and ants. In addition, the chemical is registered in California as a molluscicide and acaracide, though registrations as a fungicide, herbicide, miticide, disinfectant and repellent are currently inactive in the state. Carbaryl is effective after ingestion by the targeted pest or after absorption following bodily contact (http://extoxnet.orst.edu/pips/carbaryl.htm). Plant groups upon which carbaryl is used include citrus, fruit, vegetables, forage, forests, field crops, nuts, ornamentals, rangeland, turf and shade trees. In addition, there are significant residential, pet, lawn and garden uses. Carbaryl formulations include aqueous dispersions, baits, dusts, emulsifiable concentrates, flowables, granules, soluble concentrates, suspension concentrates, wettable powders and water dispersible granules (Crop Protection Handbook, 2009).

As a member of the carbamate class of pesticides, the action of carbaryl is based largely on its ability to inhibit acetylcholinesterase (AChE) in the nervous systems and peripheral cholinergic synapses of the target species (note: insects do not have peripheral cholinergic synapses). Carbaryl's toxicity in mammalian systems is also based on this property. Carbaryl also inhibits other cholinesterases (ChEs), including the plasma-localized butyryl ChE and the red blood cell-localized AChE. The contributions of these or other as yet unknown mechanisms to the overall toxicologic picture are currently obscure.

In contrast to the organophosphates, carbamates do not form irreversible inhibitory bonds with ChE molecules. Because of the relatively fast decarbamylation reaction, standard methods of sample preparation may underestimate the extent of peak inhibition. This is because such assays utilize extended incubation times at 37°C and large dilutions in buffer, both of which favor decarbamylation and consequent reactivation of the enzyme. More recent efforts have been made to develop ChE assay techniques that take into account the carbamate dissociation problem (Padilla and Hooper, 1992; Nostrandt *et al.,* 1993), though such techniques do not appear to have been utilized in most analyses of carbaryl-exposed tissues examined for this document. This methodological conundrum is viewed as a limitation to the interpretation of the ChE data in the present assessment. Even so, a recent assessment of carbamate-ChE interactions supports the validity of the standard Ellman assay in the carbamate case, particularly at lower doses relevant to risk assessment, while continuing to recognize its weaknesses (USEPA, 2005a).

B. REGULATORY HISTORY

Carbaryl, originally manufactured by Union Carbide and now by Bayer Crop Science, was first registered for use on cotton in the United States in 1959 (USEPA [2004]). More than 100 food tolerances are in effect for this chemical. USEPA has designated carbaryl to be a General Use Pesticide. After conducting a Special Review of carbaryl, USEPA concluded in 1980 that toxicologic concerns, particularly those relating to teratogenicity, did not warrant cancellation. A Registration Standard promulgated in 1984 and revised in 1988 specified the requirements for continued registration (USEPA, 2004).

USEPA's Interim Reregistration Eligibility Decision (IRED), issued in October 2004, cited possible health risks associated with residential and occupational exposures. This triggered mitigation directives relating to personal protective gear, engineering controls, cancellation of residential aerosol products, packaging and application requirements for residential use and restriction of residential lawn applications to spot treatment (USEPA, 2004, 2007a). The IRED also cited possible impacts on non-target organisms and endangered species that may require mitigation.

Requests for cancellation of all carbaryl uses were submitted by the Natural Resources Defence Council and the Washington Toxics Coalition after publication of the IRED . While responses by USEPA to these requests are still pending, the final Reregistration Eligibility Decision (RED), issued by USEPA in September 2007, stated "that there is a reasonable certainty that no harm will result from aggregate non-occupational exposure to the pesticide chemical residue" for currently registered uses (USEPA, 2007a). A series of data call-ins issued in March 2005 resulted in the voluntary cancellation of many products, as those registrants chose not to revise labels or conduct support studies (USEPA, 2007a). The cumulative risks from exposure to the entire N-methyl carbamate class of pesticides through food, drinking water, residential use and other non-occupational exposures were judged by USEPA "meet the safety standard set forth in section 408(b)(2) of the FFDCA" (USEPA, 2007a and 2007c).

Finally, carbaryl has been listed as a developmental toxin in males under Proposition 65 since August 7, 2009. In addition, the Office of Environmental Health Hazard Assessment announced in November 2009 its intent to list carbaryl as a carcinogen under Proposition 65 (OEHHA, 2009).

C. TECHNICAL AND PRODUCT FORMULATIONS

Carbaryl is registered in California as an insecticide, acaracide and molluscicide. Additional registrations as a fungicide, herbicide, miticide, disinfectant and repellent are currently inactive in the state. As of the last reported update of the DPR product database on Decmeber 2007, there were 53 carbaryl-containing products actively registered in California. The carbaryl concentrations in these products range between 0.126% and 97.5%. Several of these formulations contain metaldehyde in addition to carbaryl. Registered products include shampoos, dusts, powders, granulars / flakes, baits, liquids, flowable concentrates, aqueous concentrates, emulsifiable concentrates, wettable powders, soluble powders, pellets / tablets / cakes / briquets, ready-to-use liquids, impregnated materials and suspensions.

D. USAGE

According to DPR's Pesticide Use Report, agricultural use of carbaryl declined steadily, both in terms of pounds applied and acres treated, between 1994 and 2007 (Table II-1). In 2006 about 142,000 pounds of carbaryl were applied commercially, compared to 821,000 pounds in 1994. Commodities receiving the highest amounts of carbaryl in pounds applied in 2007 were oranges (27,678 lb), processing tomatoes (25,255 lb), pistachios (9453 lb), pears (9236 lb), apples (8304 lb), cantaloupe (6555 lb) and other melons (4740 lb).

Carbaryl has significant non-agricultural uses, particularly on lawns, gardens and pets. Unlike the case with agricultural applications, non-agricultural applications are not strictly quantified in California. However, DPR's Report of Pesticides Sold in California provides the total amount of carbaryl sold in the state in any given year. Comparing those values with the amounts applied in agricultural scenarios over several years gives a rough estimate of the amount of carbaryl used in non-agricultural uses. By this calculation, an average of 63% (±15%) of the total carbaryl sold was applied under agricultural conditions over the 1994-2007 period, with about 37% used under non-agricultural conditions. By way of comparison, the USEPA estimated that about half of the carbaryl sold in the United States in 1998 was used in agricultural settings and half in non-agricultural settings (though the definition of these terms was not clear) (USEPA, 2004).

Table II-1. Commercial carbaryl usage (pounds applied and acres treated) and total carbaryl sold (pounds applied) in California, 1994-2007

Year	Pounds sold [a]	Pounds applied [a] (agricultural fraction) [b]	Acres treated [b]
1994	1,264,283	820,787 (65%)	291,147
1995	1,242,400	835,811 (67%)	305,452
1996	834,427	810,162 (97%)	312,058
1997	1,142,675	754,659 (66%)	292,721
1998	506,764	427,546 (84%)	197,664
1999	639,600	388,144 (61%)	216,991
2000	563,605	364,060 (65%)	196,464
2001	412,635	286,199 (68%)	147,612
2002	421,528	256,098 (61%)	106,616
2003	329,782	205,102 (62%)	97,811
2004	388,235	240,135 (62%)	103,261
2005	412,955	190,633 (46%)	99,086
2006	411,711	156,938 (38%)	87,749
2007	323,069	142,010 (44%)	97,016

[a] Data on pounds sold are from DPR's Report of Pesticides Sold in California. Data on pounds applied and acres treated under commercial conditions are from DPR's Pesticide Use Report. Both of these reports are available at www.cdpr.ca.gov/dprdatabase.htm

[b] Percentages represent the pounds applied divided by the pounds sold for a given year. They are an estimate of the fraction that is applied commercially.

E. ILLNESS REPORTS

The following section summarizing the illness and injury reports in California to DPR is excerpted from DPR's draft Human Exposure Assessment for Carbaryl (DPR, 2010 (*in preparation*). References cited in this section are listed in that document.

Reports of illness and injury with definite, probable, or possible exposure to pesticide products are recorded in a database maintained by the Pesticide Illness Surveillance Program (PISP) at DPR. The PISP database contains information about the nature of the pesticide exposure and the subsequent illness or injury. "Definite" means that both physical and medical evidence document exposure and consequent health effects. "Probable" means that limited or circumstantial evidence supports a relationship to pesticide exposure "Possible" means that evidence neither supports nor contradicts a relationship. (DPR, 2004). Reports were summarized for illnesses associated with exposure to carbaryl alone or in combination with other pesticides for the 16-year time period, 1992-2007, inclusive (DPR, 2009). Illnesses were also classified based on types of symptoms described: systemic (symptoms such as headache, confusion, rapid pulse, dizziness, nausea); skin (symptoms such as irritation, rashes); eye (symptoms such as irritation, burning); respiratory (symptoms such as irritation, wheezing, shortness of breath). In addition to the PISP reports summarized below, a detailed listing of illnesses reported in California from 1976 through 1978 is also available (Peoples et al., 1981).

Carbaryl alone. From 1992-2007 there were 38 illness reports classified as definitely, probably, or possibly associated with the exposure to carbaryl, an average of about 2 illnesses per year. Among the symptoms reported were systemic illnesses, (including respiratory illnesses), skin injuries, and eye injuries. Individuals involved in the handling process (applicators, mixer/loaders, flaggers) reported 11 illnesses and injuries. Sixteen of the 38 illnesses/injuries resulted from agricultural carbaryl applications. There were 14 days of disability and no hospitalizations resulting from agricultural carbaryl use. For non-agricultural use scenarios there were 30 days of disability (of which 18 days are suicide-associated) and 26 days of hospitalization (23 days are suicide-associated).

Carbaryl in combination. From 1992-2007, there were 60 illnesses classified as definitely, probably, or possibly associated with exposure to carbaryl used in combination with other pesticides. For the 16-year period, this averages to 4 illnesses per year. The larger number of illnesses when carbaryl is in combination with other pesticides may be a reflection of the toxicity of the other pesticides in the mixture, or it may be that carbaryl is used more often with other pesticides than alone. Forty-eight of the 60 cases resulted from agricultural activities. Among the 48 cases, 16 illnesses and injuries occurred during the handling process (applicator, mixer/loader, flagger). There were 52 days of disability and no days of hospitalization resulting from agricultural uses. For non-agricultural uses there were 6 days of disability and no hospitalization reported.

Illnesses Outside California. There are few illness reports involving carbaryl outside California. Case histories of illnesses associated with carbaryl have been

reported from Illinois (Wiener and Young, 1995), Pennsylvania (Robinson, 1990), and New York (Dickoff et al., 1987). Most of these illnesses resulted from intentional ingestion (Dickoff et al., 1987; Robinson, 1990). One case was attributed to drift from weekly spraying by a next-door neighbor (Wiener and Young, 1995).

F. PHYSICO-CHEMICAL AND ENVIRONMENTAL PROPERTIES

Table II-2. Physico-chemical and environmental properties of carbaryl
(Note: These values are taken from DPR's Environmental Monitoring Branch reports. References can be found therein (Appendix IX).

Chemical names	1-naphthyl N-methylcarbamate; 1-naphthenol methylcarbamate; methyl carbamic acid 1-naphthyl ester
CAS registry number	63-25-2
Molecular weight	201.2
Molecular formula	$C_{12}H_{11}NO_2$
Physical state	White crystalline solid (Union Carbide in support of registration 169-058)
Melting point	142°C
Density	1.23 @ 20°C
Solubility in water	113 ppm @ 22°C, 40 ppm @ 30°C [a]
Solubility in organic solvents	methanol: 7960 ppm; hexane: 214 ppm; methylene chloride: 214,600 ppm
Vapor pressure	1.17 x 10^{-6} mm Hg 25°C
Log octanol-water partition coefficient (log K_{ow})	1.85 - 2.36
Henry's Law constant	2.74x10^{-9} atm m^3 g/mol at 25˚C
Hydrolysis half-lives	>1500 days @ pH 5; 12.1 days @ pH 7; 3.2 hr @ pH 9
Aqueous photolysis half-life	21 days (artificial light; pH 5)
Soil photolysis half-life	41 days (artificial light)
Aerobic soil half-life	4 - 17 days (sandy loam); 21-27 days (clay loam)
Anaerobic degradation half-life	78 days
Field dissipation half-life	0.76 - 10.9 days
Adsorption coefficient (K_{oc})	261

[a] A value of 120 ppm @ 30°C was reported both in The Pesticide Manual: A World Compendium (Seventh Edition, ed. by C.R. Worthing. 1983. The British Crop Protection Council. p. 88) and in The Merck Index (Thirteenth Edition, ed. by M.J. O'Neil. 2001. Merck & Co., Inc. p. 1796), implying that there is some question as to the precision of the reported values.

G. ENVIRONMENTAL FATE

The following summary was extracted from DPR Environmental Monitoring reports by Xu and by Gunasekara. These reports appear in full in Appendix IX.

Air. Carbaryl has a low vapor pressure (1.17×10^{-6} mm Hg 25°C) and low Henry's Law constant (2.74×10^{-9} atm m^3 g/mol at 25˚C), both of which lower the tendency toward volatilization. Even so, several studies have detected carbaryl in the air, even at remote sites, though the air concentrations are probably higher near the point of application. The presence of this molecule in air may be enhanced by association with particulates or spray droplets.

Water. Hydrolysis occurs rapidly at pH 7 and above; the degradation half-life is 10-17 days and 3 hours at pH 7 and pH 9, respectively (25°C), while in "acidic" water it is 1500 days (27°C). 1-naphthol, methylamine and CO_2 were identified as hydrolytic breakdown products. The presence of microorganisms is expected to enhance hydrolytic degradation. Photolysis is another degradation pathway. The photolysis half-life for carbaryl in surface water was 64 hours in spring, 52 hours in summer, 102 hours in fall and 200 hours in winter, demonstrating the contribution of sunlight to the process. The major photolysis product is 1-naphthol, which photooxidizes to 2-hydroxy-1,4-naphtho-quinone under basic conditions. Though relatively insoluble in water, carbaryl has been found in both surface water and groundwater.

Soil. Carbaryl is subject to hydrolysis, photolysis and microorganism-mediated degradation in soil. Breakdown is enhanced in aerobic, as opposed to anaerobic, soils. Moderate binding to soils is indicated by carbaryl's soil adsorbtion coefficient (K_{oc} = 100 - 600, with the precise value dependent on soil type), octanol / water partitioning (log K_{ow} = 1.85 - 2.36) and low water solubility (113 ppm at 22°C - though see footnote #1, Table II-2). Soil binding is also favored under acidic conditions. Mud has been shown to enhance the removal of carbaryl from aqueous systems, presumably by preferential binding. Carbaryl tends to be associated with the top 0 - 0.15 meters of soil, showing a dissipation half-life of 0.76 - 10.9 days. Exchangeable cations such as potassium enhance the adsorption of carbaryl to soils. Soil adsorption capacity was also enhanced by the presence of organic matter.

Biota. While some bacterial species, including *Achromobacter*, *Pseudomonas*, *Arthrobacter*, and *Xanthomonas*, and at least one fungus (*Penicillium implicatum*) can metabolize carbaryl, both it and its major metabolite 1-naphthol are toxic to such beneficial soil-dwelling microorganisms as *Chlorella vulgaris*, *Nostoc linckia* and *Synechococcus elongates*. Microbial degradation of carbaryl is enhanced under anaerobic conditions. Carbaryl is toxic to fish, aquatic invertebrates and non-target insects (honeybees), but relatively non-toxic to birds. Metabolism of carbaryl in plants is similar to that in animal systems.

III. TOXICOLOGY PROFILE

A. PHARMACOKINETICS

1. Overview

Orally administered carbaryl is excreted primarily through the urine in rats during the first 24 hours (~60-90%, depending on dose), though substantial residues appear in feces (~6-20%) and in exhaled air as CO_2 (detectable when carbaryl is labeled in the carbonyl or N-methyl carbon, but not when labeled in the naphthalene ring). The recovery kinetics in other laboratory species examined (mouse, guinea pig, sheep) appeared generally similar, though there were significant technical problems with these latter studies, which were conducted in the 1960s, used very few animals and left large fractions of the administered dose unanalyzed. A possible exception to the rat model was the dog, where approximately equal fractions were excreted in the 24-hr urine and feces, though these data suffered from similar problems.

The major metabolic pathways, which are presumably predominantly hepatic, include (1) arene oxide-mediated hydroxylation and subsequent conjugation, (2) hydrolytic decarbamylation to form 1-naphthol and subsequent conjugation, and (3) oxidation of the N-methyl moiety. Three urinary metabolites found in rat urine - 1-naphthyl glucuronide, 1-naphthyl sulfate and 4-(methyl-carbamoyloxy)-1-naphthyl glucuronide - were not found in dog urine. In addition, minimal hydrolytic product was found in the urine of a single dosed monkey. The toxicologic significance of these apparent metabolic species differences was not clear. Humans do appear to have the ability to decarbamylate carbaryl since carbaryl factory workers were shown to excrete 1-naphthyl glucuronide and 1-naphthyl sulfate, leading to speculation that humans are more similar to rats than dogs in their pharmacokinetic handling of carbaryl. However, a later study showed that intentionally dosed humans excreted only 25-30% of the carbaryl in the urine at 24 hours, suggesting that the fate of very significant fractions of the dose was unknown.

2. Pharmacokinetics in laboratory animals

Struble *et al.* (1994) studied the absorption, distribution, metabolism and excretion of ^{14}C-naphthyl-carbaryl in HSD:SD rats, 4-8 wks old, 5/sex/dose. Groups A, B and C received ~1 mg/kg carbaryl (labeled at the naphthalene-1 position), while group D received 50 mg/kg. Group A was dosed intravenously; groups B-D were dosed by oral gavage. Group C was exposed daily for 14 days with unlabeled carbaryl (1 mg/kg/day) prior to the radioactive dose. A preliminary study showed that very little label was converted to CO_2 or other volatile compounds, obviating the need to monitor these parameters in the definitive study.

Clinical signs were noted only at the high dose (tremors and prostration @ 4-6 hr, lacrimation and salivation @ 6-12 hr, languidity and swollen faces @12-24 hr, normal after 24 hr). An earlier group of group D animals dosed at 100 mg/kg were sacrificed @ 24 hr due to severe toxicity.

No gender differences in the handling of carbaryl were evident in the analyses of urine and feces. Mass balance for all dose groups ranged between 96.1% and 104%. Comparison with the intravenous group (Group A) indicated 100% absorption in all groups (one exception: Group D males registered 94.3% absorption).

Table III-1 shows the time course for excretion of the administered label. Urine was the primary route of excretion. For animals in Groups A-C (*i.e.*, animals receiving 1 mg/kg), 48.1%-55.5% of the dose appeared in the urine during the first 6 hr, with 79.64%-90.86% by 24 hr and 81.8%-92.0% by 168 hr. For Group D (animals receiving 50 mg/kg), urinary excretion was slower, 12.5%-19.3% by 6 hr, 64.4%-68.4% by 24 hr, and 77.6%-81.2% by 168 hr.

Fecal excretion was significant, though it comprised a lesser proportion of the administered dose than urinary excretion. By 168 hr, 6.98%-12.5% of the dose appeared in the feces. The fraction of the dose appearing as cage rinse/wash/wipe did not exceed 10% by 168 hr, though this fraction, which may originate as urinary or fecal “splash”, was higher in females than in males.

Tissue levels accounted for less than 0.01% of the dose after 1 week. Carcass levels accounted for less than 1% of the dose after 1 week.

Metabolites were identified by comparison to reference standards using thin-layer chromatography (TLC), high pressure liquid chromatography (HPLC) and liquid chromatography / mass spectrometry (LC/MS). Identified metabolites accounted for ~75% of the urinary radioactivity and 1% of the fecal radioactivity. The major identified fecal metabolite was dihydro-dihydroxy carbaryl. Three major metabolic pathways were elucidated: (1) arene oxide formation with subsequent metabolism to dihydro-dihydroxycarbaryl and conjugation with gluathione via the mercapturic acid pathway; (2) carbamate hydrolysis to form 1-naphthol; and (3) oxidation of the N-methyl moiety. Metabolites were conjugated with sulfate or glucuronic acid. The proposed metabolic scheme appears in Figure 1.

This study was considered acceptable by FIFRA standards. [1]

[1] This risk characterization document contains technical references to the acceptability, non-acceptability or supplemental quality of the studies used to gauge risk. These designations refer to each study's status with regard to guidelines established through the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). In this context, a "supplemental" designation indicates that the work was not done using those guidelines. It should be emphasized that DPR does not necessarily base its judgement of the usefulness of a study for risk assessment purposes on the FIFRA designation. More to the point, a supplemental or unacceptable study can play an important or even critical role in the ultimate risk characterization.

Table III-1a Excretion time course for ^{14}C-carbaryl administered intravenously (Group A) and by oral gavage (Groups B-D); male HSD:SD rats (Struble *et al.* [1994])

Collection interval (hr)	Feces (% of total dose)				Urine (% of total dose)				Cage rinse/wash/wipe (% of total dose)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0-6	0.78± 1.729	nd[a]	nd	0.04± 0.042	55.5± 8.17	49.6± 8.54	51.5± 16.56	19.3± 3.73	nd[a]	nd	nd	nd
6-12	6.21± 2.905	4.88± 1.453	4.39± 0.744	1.26± 2.809	23.7± 11.09	29.7± 8.21	31.8± 16.45	22.9± 1.85	nd	nd	nd	nd
12-24	2.32± 0.578	3.70± 0.76	3.60± 1.731	7.93± 2.246	4.81± 0.943	7.56± 1.272	7.56± 1.804	26.2± 5.11	2.92± 1.344[b]	3.50± 2.091[b]	2.49± 1.071[b]	5.81± 2.555[b]
0-24 [d]	9.31	8.58	7.99	9.23	84.01	86.86	90.86	68.40				
24-48	0.67± 0.491	0.40± 0.114	0.49± 0.144	2.82± 1.254	1.14± 0.423	0.88± 0.255	0.84± 0.215	7.65± 1.916	nd	nd	nd	nd
48-72	0.07± 0.032	0.05± 0.011	0.06± 0.047	0.27± 0.118	0.22± 0.164	0.13± 0.056	0.11± 0.030	0.62± 0.236	nd	nd	nd	nd
72-96	0.04± 0.017	0.02± 0.010	0.01± 0.009	0.09± 0.052	0.11± 0.082	0.06± 0.022	0.05± 0.009	0.32± 0.174	nd	nd	nd	nd
96-120	0.05± 0.040	0.01± 0.013	<0.01	0.04± 0.013	0.09± 0.102	0.04± 0.024	0.05± 0.027	0.30 ±0.330	nd	nd	nd	nd
120-144	0.02± 0.015	<0.01	0.01± 0.022	0.03± 0.019	0.08± 0.060	0.04± 0.025	0.03± 0.013	0.19± 0.149	nd	nd	nd	nd
144-168	0.02± 0.015	<0.01	<0.01	0.02± 0.007	0.06± 0.038	0.06± 0.076	0.02± 0.013	0.15± 0.097	0.72[c]	0.46[c]	0.48[c]	1.01[c]
Total	10.2± 2.65	9.06± 1.157	8.57± 0.992	12.5± 2.05	85.7± 3.62	88.1± 1.98	92.0± 3.10	77.6± 4.61	3.64± 1.356	3.96± 2.137	2.97± 1.197	6.82± 2.778

Note: Group A, 1 mg/mg ^{14}C-carbaryl, iv; Group B, 1 mg/mg ^{14}C-carbaryl, oral gavage; Group C, 1 mg/mg ^{14}C-carbaryl oral gavage after 14 days of 1 mg/mg/day unlabeled carbaryl, oral gavage; Group D, 50 mg/mg ^{14}C-carbaryl oral gavage.

[a] Not determined. For the cage rinse/wash/wipe determinations, the value represents the percent retrieved at the latter time point only.
[b] Cage rinse only.
[c] Cage wash + cage wipe (combined for simplicity; no standard deviation).
[d] For the time 0-24 hr interval, the percentages for the previous intervals were added together by the risk assessor. Consequently, no standard deviations were presented.

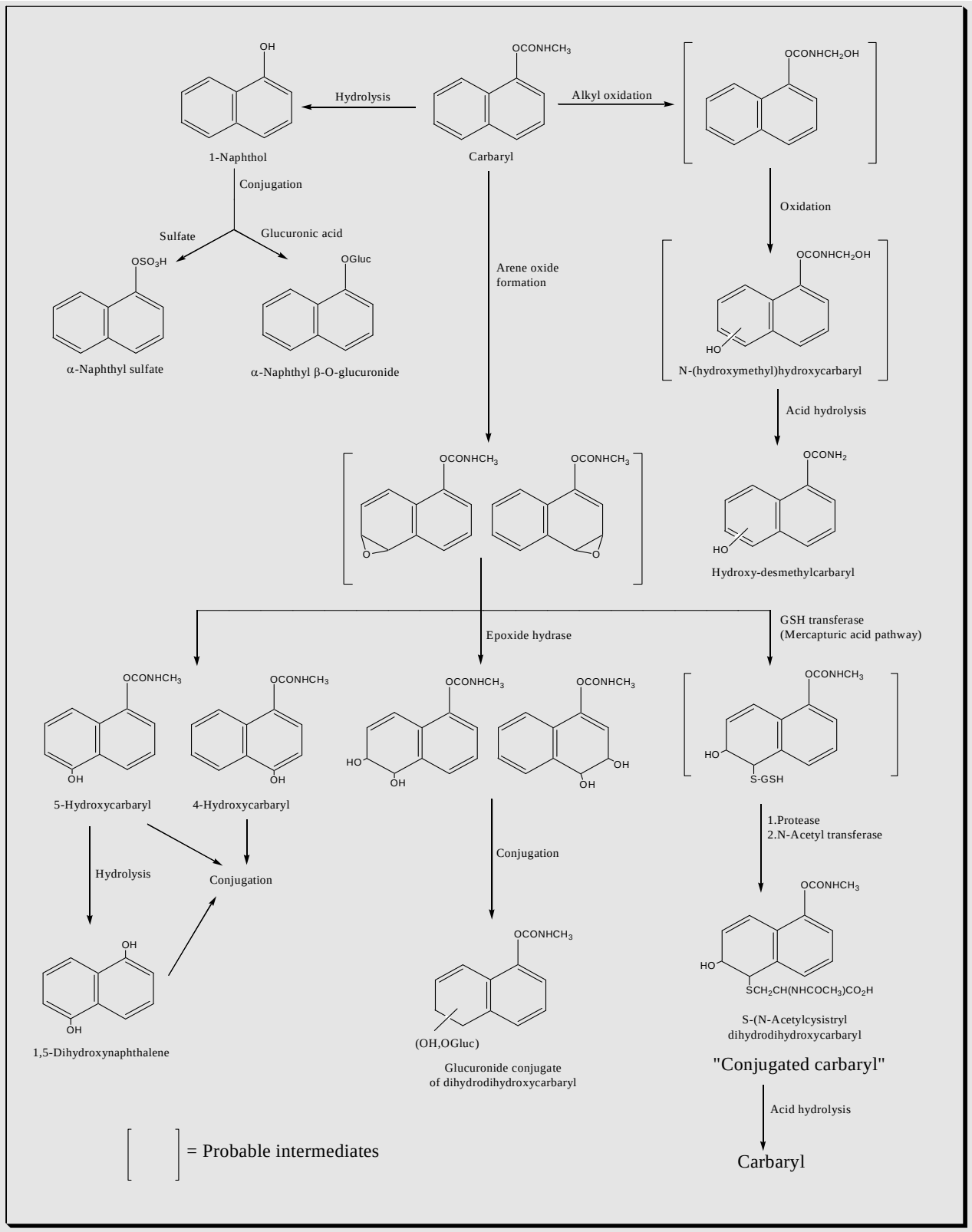
Table III-1b. Excretion time course for ^{14}C-carbaryl administered intravenously (Group A) and by oral gavage (Groups B-D); female HSD:SD rats (Struble *et al.* [1994])

Collection interval (hr)	Feces (% of total dose)				Urine (% of total dose)				Cage rinse/wash/wipe (% of total dose)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0-6	nd[a]	nd	0.10± 0.222	0.11±0 .110	48.5± 3.57	48.1± 6.74	53.4± 6.33	12.5± 5.95	nd[a]	nd	nd	nd
6-12	5.15± 2.007	3.87± 2.704	2.93± 2.780	0.10± 0.233	26.7± 8.12	23.6± 4.68	21.6± 4.32	21.4± 6.49	nd	nd	nd	nd
12-24	2.73± 2.440	3.97± 1.671	3.95± 2.173	1.92± 2.843	5.66± 1.846	7.94± 3.522	8.09± 2.256	30.5± 4.79	4.54± 3.196[b]	9.04± 4.288[b]	9.56± 2.292[b]	6.46± 2.763[b]
0-24	7.88	7.84	6.98	2.13	80.86	79.64	83.09	64.40				
24-48	0.51± 0.297	0.39± 0.143	0.52± 0.232	3.98± 1.651	1.52± 0.798	1.64± 0.792	1.36± 0.602	14.2± 7.49	nd	nd	nd	nd
48-72	0.13± 0.088	0.06± 0.024	0.06± 0.024	0.60± 0.285	0.38± 0.229	0.23± 0.083	0.19± 0.061	1.67± 1.189	nd	nd	nd	nd
72-96	0.06± 0.019	0.03± 0.013	0.04± 0.015	0.13± 0.055	0.20± 0.095	0.14± 0.060	0.10± 0.037	0.43± 0.280	nd	nd	nd	nd
96-120	0.06± 0.037	0.06± 0.095	0.03± 0.021	0.05± 0.031	0.15± 0.104	0.08± 0.047	0.07± 0.012	0.19± 0.151	nd	nd	nd	nd
120-144	0.04± 0.016	0.02± 0.011	0.04± 0.048	0.06± 0.043	0.11± 0.055	0.07± 0.038	0.06± 0.044	0.17± 0.130	nd	nd	nd	nd
144-168	0.03± 0.013	<0.01	<0.01	0.03± 0.015	0.09± 0.035	0.06± 0.041	0.04± 0.013	0.12± 0.082	0.78[c]	0.65[c]	0.44[c]	0.49[c]
Total	8.71± 3.430	8.40± 1.562	7.68± 0.785	6.98± 1.222	83.3± 3.12	81.8± 5.38	85.0± 1.68	81.2± 2.50	5.32± 3.085	9.69± 4.231	10.0± 2.32	6.95± 1.75

Note: Group A, 1 mg/mg ^{14}C-carbaryl, iv; Group B, 1 mg/mg ^{14}C-carbaryl, oral gavage; Group C, 1 mg/mg ^{14}C-carbaryl oral gavage after 14 days of 1 mg/mg/day unlabeled carbaryl, oral gavage; Group D, 50 mg/mg ^{14}C-carbaryl oral gavage.

[a] Not determined. For the cage rinse/wash/wipe determinations, the value represents the percent retrieved at the latter time point only.
[b] Cage rinse only.
[c] Cage wash + cage wipe (combined for simplicity; no standard deviation).
[d] For the time 0-24 hr interval, the percentages for the previous intervals were added together by the risk assessor. Consequently, no standard deviations are presented.

Figure 1. Proposed metabolic pathways for carbaryl (*from* Struble *et al.* [1994])



Totis (1997) conducted a pharmacokinetics study to "investigate the mechanisms that caused the appearance of an increased incidence of tumours during the final year of a chronic dietary feeding study in the rat at the high dose level of 7500 ppm. For this purpose, a series of experiments were performed using the 15 month old male [CD] rat."

There were 5 experimental groups: Group A, single gavage administration at the same high dose level (~50 mg/kg ^{14}C-carbaryl, labeled in the naphthylene ring) used in the Struble *et al.* (1994) study; Group B, 0 ppm (control) dietary administration group; Group C, 250 ppm dietary administration group; Group D, 7500 ppm dietary administration group; Group E, 1500 ppm dietary administration group (this group was done later than the other groups). Groups B-E were dosed at the indicated dietary level for 83 days (except where indicated) followed by a week of gavage administration with 2 mg/kg/day ^{14}C-carbaryl. Group A comprised 5 animals. Groups B-E comprised 25 animals (5/group used for mass balance, 5/group for urinary / fecal metabolism identification, 10/group used for metabolism identification in tissues, 5/group dosed with dietary carbaryl for 90 days and used for histopathology and enzyme activity determinations). The achieved doses in groups C, D and E were 9.89, 250.71 and 58.96 mg/kg/day, respectively, over 13 weeks.

Body weights were significantly less than controls at 7500 ppm on days 14, 29 and 83 (day 83 mean weights, 0 ppm: 767.1 g; 7500 ppm: 613.9 ppm**; $p<0.01$), with an increase in liver, spleen and thyroid weights (absolute and relative to body weight). Liver histopathology indicated centrilobular hypertrophy, pericholangitis and a tendency toward bile duct hyperplasia at 7500 ppm. Liver glutathione concentration was also elevated at 7500 ppm (46.8 vs. 94.40** µmol/g liver @ 0 and 7500 ppm, respectively; **$p \leq 0.01$). Thyroid follicular cell hypertrophy was noted in 0/5, 3/5, 5/5 and 5/5 rats at 0, 250, 1500 and 7500 ppm. Kidney transitional cell hyperplasia was noted in 0/5, 0/5, 1/5 and 2/5 rats at those doses.

In the dietary administration groups (Groups B-E), 64-90% of the administered dose was excreted in the urine within the first 24-48 hr (the 7500 ppm group had the lowest urinary excretion levels), with 8-18% in feces. For the single dose 50 mg/kg group, 63% had appeared in the urine and 5% in feces by 48 hr. There were 23 metabolites in urine and twenty in feces, including carbaryl. The major urinary metabolites were UMET/11 (glucuronide of dihydro-dihydroxy carbaryl), UMET/18 (α-naphthyl-β-D-glucuronide, sodium salt) and UMET/23 (sulfo conjugate of naphthol). The appearance of UMET/11 in the urine increased at 1500 and 7500 ppm, while UMET/23 (sulfo-conjugate of the naphthol) decreased, particularly at 7500 ppm. Tissue levels were low, with the kidneys generally containing the most residual activity (though even kidney levels were less than 1% of the administered dose). It was concluded that 15-18 month male rats are capable of significant metabolism of carbaryl, similar to the young rats studied by Struble *et al.* (1994).

As this study was not executed according to FIFRA guidelines, it was considered to be supplemental.

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Similar to the Totis (1997) study in rats, Valles (1999) initiated a study in CD_1 mice to "investigate the contribution of metabolism to the mechanisms that resulted in the appearance of an increased incidence of tumours during the final year of a chronic dietary feeding study [in mice] at the dose level of 1000 and 8000 ppm". Males (10/dose) were 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 (labeled at the naphthalene-1 position) on day 15. Assuming a food consumption rate of ~6 g/day and body weights of about 0.03 kg, these doses corresponded to carbaryl doses of approximately 0, 2, 20, 200 and 1600 mg/kg/day. Urine and feces were collected at 24-hr intervals for a total of 168 hr following dosing, after which the animals were sacrificed.

Radioactivity in the carcass and blood was also determined. The metabolites in pooled urine were quantified for 0-24, 24-48 and 48-96 hr.

Urine was the major excretory route. Within the first 24 hr, 45-59% of the dose appeared in the urine. By 48 hr and 168 hr, it had climbed to 53-68% and 55-70%, respectively. If cage washes were added to urine (on the assumption that the radioactivity in this fraction originated as urinary "splash"), the total urinary excretion by 168 hr was 83.55% (0 ppm), 72.71% (10 ppm), 80.73% (100 ppm), 84.17% (1000 ppm) and 79.41% (8000 ppm). Fecal excretion accounted for 12-19% of the dose by 168 hr.

Twenty-one metabolites were detected in the urine. The four major metabolites, found in all dose groups, were: (1) dihydro, dihydroxynaphthyl sulfate, (2) hydroxycarbaryl glucuronide, (3) α-naphthyl sulfate and (4) α-naphthyl β–D glucuronide. Three of these (#2-4) had been identified in 15-18 month old male rats by Totis (1997), suggesting that mice metabolized carbaryl in a manner that was qualitatively similar to the rat, but with some quantitative differences. There was a shift in the urinary metabolite pattern at 8000 ppm, with increases in (1) and (2) above, which are apparently formed by epoxide intermediates. Therefore, high doses of carbaryl could alter the metabolism, distribution and excretion patterns for this compound. The authors considered it plausible that such a metabolic transition at high doses could account for the oncogenicity of this compound in mice (see Hamada [1993b], summarized below in section III.D.2.) and in rats (see Hamada [1993a], summarized below in section III.D.2.).

This study was deemed supplemental.

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Krolski *et al.* (2003a) investigated the pharmacokinetic behavior of carbaryl after exposure by the oral, dermal and intravenous (iv) routes. Thirty two male Sprague-Dawley rats / group were treated as indicated: (1) oral gavage with either 1.08 mg/kg [naphthyl-1-^{14}C]-carbaryl or 8.45 mg/kg [naphthyl-4a,5,6,7,8,8a-^{14}C]-carbaryl; (2) dermal, up to 10 hr exposure with either 17.25 mg/kg [naphthyl-1-^{14}C]-carbaryl or 102.95 mg/kg [naphthyl-4a,5,6,7,8,8a-^{14}C]-carbaryl; (3) iv injection with either 0.80 mg/kg [naphthyl-1-^{14}C]-carbaryl or 9.20 mg/kg [naphthyl-4a,5,6,7,8,8a-^{14}C]-carbaryl. For the oral and dermal routes, 4 animals / time point were euthanized at 15 and 20 min and at 1, 2, 4, 6, 12 and 24 hr post dose. For the iv route, 4 animals / time point were euthanized at 5, 10, 20 and 30 min and at 1, 2, 4 and 8 hr post injection. Total radioactive residues (TRR) were determined in the whole blood, plasma, RBCs and brain of all animals. Liver and fat tissue from high dose animals were also assayed for TRR. Composite samples were analyzed for parent compound or specific metabolites. Urine and fecal samples were not collected.

Peak levels of radioactivity were detected in the blood at 15 and 30 min for the low and high dose oral treatments, respectively; at 4 and 12 hr for the dermal applications; and were already maximal by the first time point (5 min) for the iv injections. *Oral dosing*: by 24 hr, radioactivity levels had decreased to 0.81%-2.4% of peak levels in blood fractions (both doses), 0.60%-2.4% in brain (both doses), 0.67% in liver (high dose only) and 0.32% in fat (high dose only). *Dermal dosing*: by 24 hr, radioactivity levels had decreased to 15.9%-25.8% of peak levels in blood fractions (both doses), 27.1%-30.6% in brain (both doses), 24.4% in liver (high dose only) and 15.6% in fat (high dose only). *Iv dosing*: by 24 hr, radioactivity levels had decreased to 4.6%-10.5% in blood fractions (both doses), 1.1%-1.3% in brain (both doses), 5.7% in liver (high dose only) and 0.72% in fat (high dose only).

Metabolic analysis revealed that carbaryl was rapidly degraded through hydrolysis of the carbamate ester linage, as indicated by the recovery of more polar compounds, 1-naphthol and 1-naphthol sulfate in the plasma. N-hydroxy-carbaryl was recovered as a minor metabolite in the brain. By 24 hr post oral dose and 8 hr post injection dose the carbaryl level in the brain had

fallen to 0.4 and 0.1% of the peak levels, respectively. Similar metabolic patterns were seen in the liver and fat.

As this study was not conducted according to FIFRA guidelines, it was considered to be supplemental.

Krolski *et al.* (2003b) extended the above study by exposing Sprague-Dawley rats simultaneously by the oral and dermal routes. Twenty males received two gavage doses of 0.085 mg/kg; there was a 1-hour interval between the doses. Concomitantly, a 2-hour dermal exposure of 0.871 mg/kg was also executed. The test material was [naphthyl-4a,5,6,7,8,8a-^{14}C]-carbaryl. Four animals per time point were euthanized at 0.25, 0.5, 1, 3 and 5 hours after the second oral dose. Total radioactive residues (TRR) were determined in whole blood, plasma, RBCs and brain of all animals.

Peak levels of radioactivity occurred in the blood and brain 15 minutes after the second oral dose (*i.e.*, while the dermal exposure was still occurring), though no measurements were taken during the first hour. A slight upward inflection of the TRR *vs.* time curve for whole blood may reflect a contribution from the dermal component, though this could not be verified [2]. Analysis of the metabolites in the brain revealed that carbaryl was degraded through hydrolysis of the carbamate ester linkage as indicated by the recovery of more polar metabolites, 1-naphtol and 1-naphthol sulfate.

As this study was not conducted according to FIFRA guidelines, it was considered to be supplemental.

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Thomas (1994) initiated a study "to identify and phenotype a prospective cytochrome p-450 inducing potential of carbaryl in the livers of male CD1 mice following dietary administration of the test article at a dose level of 8000 ppm (8000 mg/kg diet) for 14 consecutive days". This study was part of a larger study which examined carbaryl's potential for DNA damage (or chromosomal aberrations; see section III.G.). Frozen livers were thawed, homogenized and cytosolic and microsomal fractions obtained by centrifugation. Body weights in treated mice were 85% of controls (28.88 g *vs.* 34.08 g; $p<0.001$). Relative liver weights were increased to 135% of controls (6.47% *vs.* 4.79%; $p<0.01$). Microsomal protein was increased to 132% of controls (22.75 mg/g liver *vs.* 17.23 mg/g liver; $p < 0.01$). Cytochrome p-450 was elevated 1.3-fold over controls (15.13 nmol/min/g liver *vs.* 11.21 nmol/min/g liver; $p<0.05$), 7-ethoxyresorufin o-de-ethylase (EROD) 1.9-fold over control (4.09 nmol/min/g liver *vs.* 2.15 nmol/min/g liver; $p<0.05$), 7-pentoxyresorufin o-de-ethylase (PROD) 3.1-fold over control (0.655 nmol/min/g liver *vs.* 0.209 nmol/min/g liver; $p<0.01$), and total testosterone hydroxylation 1.52-fold over control (86.59 *vs.* 56.95 nmol/min/g liver; $p<0.05$). The slightly increased level of glutathione did not reach statistical significance. Carbaryl was considered to be a weak barbiturate-type inducer of cytochrome p-450 in male mice.

This study was considered to be supplemental.

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The following three paragraphs summarize three older metabolism studies from Knaak *et al.*

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[2]Comparison with the dermal regimen in Krolski *et al.* (2003a) at 17.25 mg/kg showed the blood peak occurring at 4 hours in that study, with little evidence of a contribution at 1 hour. The exposure regimen in that study continued for 10 hours, unlike the current study where exposure was discontinued after 2 hours.

(1965, 1967, 1968) in several species. Knaak's rat data largely support Struble (1994), though it appears that the dog may excrete a higher proportion of the naphthyl residues in the fecal fraction and may not produce certain metabolites in the urine (Knaak, 1967). However, Knaak's studies used low animal numbers and did not analyze the metabolites in large fractions of the total dose, particularly in the feces.

Knaak *et al.* (1965) studied the metabolism of carbaryl in the rat and guinea pig after intraperitoneal administration of carbaryl-naphthyl-^{14}C, carbaryl-methyl-^{14}C or carbaryl-carbonyl-^{14}C. When 4 rats each (sex not stated) were dosed by gavage with 20 mg/kg of these compounds, an average of 94% was excreted over a 7-day period, with excretion essentially complete in 3 days. The approximate percent of dose in urine / feces / CO_2 after 4 days was, for carbaryl-naphthyl-^{14}C: 72% / 10% / 0%; for carbaryl-methyl-^{14}C: 69% / 7% / 11%; for carbaryl-carbonyl-^{14}C: 47% / 8% / 32%. As might be expected, carbaryl-naphthyl-^{14}C was not detected as $^{14}C\,O_2$, while carbaryl-methyl-^{14}C and carbaryl-carbonyl-^{14}C produced CO_2 at 11% and 32% of the dose, respectively. Two to 3% of the methyl-^{14}C dose was recovered in the intestinal tract, carcasses and remaining organs (neither naphthyl-^{14}C nor carbonyl-^{14}C residues were detected in tissues). Recovery data for guinea pigs were not presented.

Urinary metabolites of carbaryl-naphthyl-^{14}C, carbaryl-methyl-^{14}C and carbaryl-carbonyl-^{14}C (rat only) were examined in the rat and guinea pig using DEAE-cellulose and thin layer chromatography. Pooled samples collected during the first 24 hours after intraperitoneal injection of 3 mg in 300 mg of polyethylene glycol 400 to each of 3 male rats and 3 guinea pigs (sex not stated) were examined. In the rats, the 24-hr samples yielded 73%, 47% and 48% of the naphthyl, methyl and carbonyl doses, respectively [3], while in the guinea pigs the naphthyl and methyl ligands yielded 85% of the dose (separate values for each ligand were not reported for guinea pigs). The following urinary metabolites were identified: 1-naphthyl methylcarbamate N-glucuronide (guinea pig only), 1-naphthyl methylimidocarbonate O-glucuronide (the most prominent identifiable metabolite of all three compounds in rat urine at 26.0-45.3% of the recovered ^{14}C and the most prominent identifiable metabolite of carbaryl-methyl-^{14}C in guinea pig urine at 30.1% of the recovered ^{14}C), 4-(methylcarbamoyloxy)-1-naphthyl glucuronide, 1-naphthyl glucuronide (the most prominent metabolite of carbaryl-naphthyl-^{14}C in the guinea pig at 26.5% of the recovered dose), 4-(methylcarbamoyloxy)-1-naphthyl sulfate, 1-naphthyl sulfate, unidentified neutrals, and two unidentified metabolites (one of which was found only in the guinea pig).

Rat and guinea pig liver microsomes incubated with carbaryl-naphthyl-^{14}C in the presence of a hydrogen donor ($NADPH_2$) and uridine diphosphoglucuronide (UDPGH) formed a spectrum of metabolites. These included unidentified water-soluble neutrals, 4-(methylcarbanoyloxy)-1-naphthyl glucuronide, 1-naphthyl glucuronide and two unidentified metabolites (one of which was found only in the rat system). The only major urinary metabolites not formed by the liver preparations were 4-(methylcarbamoyloxy)-1-naphthyl sulfate and 1-naphthyl sulfate.

Fluorescence chromatograms were conducted on 24-hr pooled urine samples from men

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[3] The 21% discrepancy for the carbaryl-methyl-^{14}C, 4-day urinary value reported in the recovery experiment in the first paragraph was unexplained and only partially accounted for by the difference in collection time: 24 hr in the metabolite experiment *vs.* 4 days in the recovery experiment (recovery at 24 hr in the latter experiment was ~69%). Based on animal weights of ~150 g, the doses for the two experiments (20 mg/kg in the recovery experiment, 300 mg/animal in the metabolite experiment) were equivalent, so could not account for the discrepancy.

exposed to carbaryl dust in a packaging operation at a Union Carbide plant (though the number of men was not stated). The only detectable metabolites were 1-naphthyl glucuronide (~25 µg/ml) and 1-naphthyl sulfate (~5 µg/ml). These data demonstrated that humans can hydrolyze and conjugate carbaryl. The apparent absence of other metabolites may be a function of the sensitivity or timing of the fluorescence assay.

In conclusion, as stated in the report (p. 542-3), "Carbaryl is metabolized in the rat and guinea pig to a series of eight or more water-soluble compounds. Forty-seven to 57% of the metabolites excreted possess the intact C-O-C(O)N-C structure, indicating that a nonhydrolytic pathway exists for carbaryl.... Thirty-nine to 44% of the administered carbaryl was hydrolyzed and the liberated 1-napththol conjugated with glucuronic and sulfuric acids." [4] In addition, the study confirmed the ability of humans to hydrolyze (decarbamylate) and conjugate carbaryl.

While this study contains useful data, the intraperitoneal exposure route may not be representative of oral, dermal or inhalation exposure. Furthermore, (1) the sex of the animals was not always identified in this study, (2) there were relatively few animals tested, and (3) the 24-hr urine samples did not account for large portions of the initial dose. As a result, much of the metabolic picture in rats and guinea pigs (not to mention humans) was not characterized by the study. This study was considered to be supplemental.

Knaak *et al.* (1968) studied the metabolism of carbaryl in the monkey (1 female rhesus), pig (2 females) and sheep (1 female) after administration of either carbaryl-naphthyl-^{14}C or carbaryl-methyl-^{14}C. The monkey received a dose of 300 mg/kg. The pigs and the ewe received a dose of 25 mg/kg. In addition, two human males received a 2 mg/kg dose of unlabeled carbaryl. Doses were administered orally in gelatin capsules. Urinary metabolites were elucidated by DEAE-cellulose ion exchange chromatography.

The pigs excreted 83.4% and 1.6% of the naphthyl label in the urine and feces, respectively, within 5 days of oral administration in gelatin capsules. The parallel study with the methyl label resulted in 70% and 1% appearing in urine and feces. Results for the ewe were 71.4% and 3.4% for the naphthyl label and 62.4% and 5.4% for the methyl label after 4 days. Humans excreted about 25-30% of the carbaryl in the urine within 24 hr, with very little excretion thereafter, as determined by fluorescence chromatography. Recovery data were not reported for the monkey.

A spectrum of metabolic products resulting both from hydroxylation and hydrolysis of carbaryl were noted in the 24-hr urine samples of all species tested. In the pig, two major metabolites possessing the intact carbamate structure (*i.e.*, C-O-C(O)-N-C), including 1-naphthyl methylimidocarbonate O-glucuronide (compound D: 38-46% of total ^{14}C recovered from the column) and 4-(methylcarbamoyloxy)-1-naphthyl glucuronide (compound F:15-16%), were recovered. In addition, unidentified neutrals (referred to as compound A, probably including parental carbaryl and naphthol: 10-23%) and one hydrolysis product, 1-naphthyl glucuronide (compound G: 5.5%) were also detected. The ewe excreted five intact carbamates: compounds D (26-42%), F (13-24%), H (4-(methylcarbamoyloxy)-1-naphthyl sulfate: 4-13%), and two unidentified intacts not identified in rat, guinea pig, monkey or pig urine (compound J: 6.9% and compound K: 3-9%). Also identified in ewe urine were neutral compound A (3-14%), and two hydrolysis products, compounds G (11.9%) and I (1-naphthyl sulfate: 25.2%). The

[4] Calculations by the risk assessor (A. Rubin) did not precisely verify these values. In the 24-hr pooled rat urine samples, carbamate-intact metabolites from the three ligands accounted for 47%-68% of the total radioactivity, while in the guinea pig such metabolites from the two ligands accounted for 47%-67% of the total radioactivity.

monkey excreted three intact carbamates, including compounds D (16-18%), F (31-38%) and H (14-26%), in addition to neutral compound A (16-17%). Virtually no hydrolyzed metabolites (*i.e.*, compounds G or I) were excreted in monkey urine.

The following urinary metabolites were detected in male humans: unidentified neutrals (compound A, not quantitated), an unidentified metabolite (compound C, not quantitated), compound D (not quantitated), compound F (4-6%), compound G (10-16%), compound H (0% - interpreted as trace), and compound I (6-11%). These results confirmed the ability of humans to hydrolyze the carbamate moiety (*i.e.*, decarbamylate) observed by Knaak *et al.* (1965). However, in view of the small fraction of the total dose appearing in the human 24-hr urine samples (~30%, as noted in the second paragraph above), it is difficult to draw conclusions concerning the overall metabolite profile in humans. The monkey showed little tendency to hydrolyze carbaryl; the pig had somewhat greater tendency, but less than sheep or humans. The metabolite profiles for rat and man appeared qualitatively similar, with the caveat that only a fraction of the human excretion profile was analyzed.

Some data from this study may be useful, though very few animals were tested and the 24-hr urine samples did not account for sufficient portions of the initial dose, particularly in humans. As a result, much of the metabolic picture remained unclear for these species.

This study was considered to be supplemental.

Knaak and Sullivan (1967) studied the metabolism of carbaryl in three female beagles. Each animal was dosed successively (one week apart) with carbaryl-naphthyl-^{14}C and carbaryl-methyl-^{14}C. The dose for each ligand was 25 mg/kg. Urine and feces were collected over a 7-day period. Urinary metabolites were analyzed in first day urine samples by ion exchange, thin layer chromatography and fluorometry.

Excretion was essentially complete by 4 days. For carbaryl-naphthyl-^{14}C, about 38% of the dose was excreted in the urine and ~35% in the feces. Thus ~73% of the dose was excreted by those routes in that time. For carbaryl-methyl-^{14}C, about 21% of the dose appeared in the urine and ~11% in the feces, resulting in about 32% of the dose excreted by those routes. The unequal distribution of these two labels was interpreted as evidence that an N-methyl hydrolytic pathway exists in the dog. The presence of essentially equivalent amounts of carbaryl-naphthyl-^{14}C in the feces as in the urine presents a quantitative difference from the rat, which excretes less than 10% of the carbaryl-naphthyl-^{14}C in the feces compared to 77-92% in the urine after 1 week (Struble *et al.* [1994]; Knaak [1965] shows approximately the same proportions).

Three important urinary metabolites normally found in rat urine were not found in the dog: 1-naphthyl glucuronide, 1-naphthyl sulfate and 4-(methyl-carbamoyloxy)-1-naphthyl glucuronide. Most other rat metabolites (see the studies summarized above) were also found in dog urine. As stated in the report (p. 1126): "...the major difference between the rat and dog appears to be the inability of the dog to liberate 1-naphthol or hydroxylate carbaryl. The dog can conjugate naphthol, and appears to conjugate carbaryl directly." The latter statement may have referred to the three unidentified ^{14}C-naphthyl peaks, labeled E, F and H in this study (but not corresponding to those in Knaak *et al*. [1965]), though this is not explicitly stated.

While it may be true, as stated in this study, that dogs metabolize carbaryl differently than rats (or humans, for which there are even less data), and may excrete more of the naphthyl group in the feces, the usefulness of this study was limited by its small scope. Very few animals were used (understandable in view of the species) and the metabolites from an appreciable fraction of the dose (most prominently, from the large fecal fraction) were not characterized. In the absence of a more contemporary dog study, it cannot be used to disqualify this species as a laboratory subject in the characterization of human risk from carbaryl.

This open literature article was considered to be supplemental.

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Rickard and Dorough (1984) investigated the possibility that the *N*-nitroso derivatives of carbamate pesticides could be formed under the acidic conditions of the stomach. In the *in vivo* experiments, female Sprague-Dawley rats and Hartley guinea pigs were treated by gavage with ^{14}C-carbofuran or ^{14}C-carbaryl and sodium nitrite (controls consisted of animals treated with carbamate alone). The stomach contents were removed from the animals, processed and analyzed by two-dimensional thin layer chromatography using nitrosocarbamate standards. *In vitro* experiments were conducted by incubating the sodium nitrite and radiolabeled carbamates with stomach contents.

Guinea pigs formed nitrosocarbamates more readily than rats: 1.54% of the carbaryl dose and 0.65% of the carbofuran dose were detected as the *N*-nitroso derivative in guinea pigs *vs.* 0.02% and 0.03% in the rat, respectively. When the incubations were carried out *in vitro* using isolated stomach contents, 37.4% of the carbaryl dose and 18.9% of the carbofuran dose were detected as the *N*-nitroso derivative in guinea pigs *vs.* 0.57% and 0.31% in the rat, respectively. This species difference was attributed to the lower pH of the guinea pig stomach (1.2-1.6) *vs.* the pH of the rat stomach (3-5), a conclusion which was supported by an experiment in which the incubation with carbaryl was performed after the pH of the rat stomach was artificially lowered with HCl or acetic acid. As the guinea pig stomach pH approximates that of the human, this supports the possibility that nitrosocarbamates may be formed readily in the human stomach. The low nitrosocarbamate *in vivo* yields in either species were considered to reflect the instability of the derivatives, as well as the rapid absorption of both the parent compound and the derivative.

The toxicologic significance of nitrosocarbaryl formation is not clear. This open literature article was considered to be supplemental.

B. ACUTE TOXICITY (including ACUTE NEUROTOXICITY)

1. Overview

The acute toxicity of carbaryl results from its ability to carbamylate, and thus inhibit, acetyl cholinesterase (AChE) at synapses and neuromuscular junctions. Resulting local accumulations of acetylcholine (ACh) generate cholinergic effects, including tremors, sluggishness, epigastric pain, blurred vision, nausea, sweating, lassitude, salivation, piloerection and lacrimation. The lower LD_{50} reported for the intraperitoneal route than for the oral route in rodent studies implies that hepatic (or possibly gastrointestinal) metabolism and excretion plays an important mediating role in the organismal response to carbaryl. The critical LOEL for oral toxicity was 1 mg/kg, based on cholinergic effects in rats at that dose in a developmental neurotoxicity study (Robinson and Broxup, 1997; summarized in section III.H). Benchmark dose analysis was used to arrive at an oral LED_{10} of 0.25 mg/kg. The critical LOEL for inhalation toxicity was 10 mg/m^3, based on inhibition of brain cholinesterase activity at that dose in rats after a 3-hr exposure (Weinberg, 2008). Benchmark dose analysis was used to arrive at an inhalation LED_{10} of 5.5 mg/m^3, equivalent to an internal dose of 0.66 mg/kg assuming a default breathing rate of 0.96 m^3/kg/day. According to Baron (1991), only one human death, a suicide, had been unambiguously tied to carbaryl ingestion by that time. Even in that case, the mortality may have resulted from use of antidotal 2-PAM. A detailed medical account of a near suicide considered the possibility that carbaryl could have long-term neuropathic sequelae in humans similar to those seen for organophosphates (Dickoff *et al.*, 1987). The reported effects resulting from an extended accidental exposure in an older male support this notion (Branch and Jacqz, 1986).

Nonetheless, owing to the instability of the carbamate-AChE bond, recovery from acute effects is expected in most cases when exposures are low or moderate.

2. Human exposures

Baron (1991) reviewed several experimental studies of systemic carbaryl exposures in humans. No effects were observed in one acute oral study in men at doses as high as 2 mg/kg. In another study, a scientist investigating possible antihelmintic properties of carbaryl, ingested approximately 2.8 mg/kg (250 mg total). Epigastric pain followed by profuse sweating began after 20 minutes, followed by lassitude and vomiting. Recovery was evident by one hour (3 mg of atropine were ingested by that time), and complete by 2 hours.

Baron described a similar incident as follows: “A scientist ingested, on an empty stomach, a suspension containing about 420 mg of carbaryl (5.45 mg/kg). (He had previously taken larger doses about an hour after a meal without any resulting illness.) No symptoms appeared for 80 min. After 85 min, he noticed a slight change in vision lasting for 15-20 min. After 90 min, he began to feel nauseated and lightheaded; 2 mg of atropine helped, but the symptoms returned. By 17 min, after the onset of symptoms, he had taken 4.8 mg of atropine, despite which he began to sweat very profusely. Hyperperistalsis developed (with little pain). Nausea persisted for about 2 hr, but without vomiting or diarrhea. He experienced a profound sense of weakness and preferred to remain perfectly still, but had no difficulty in breathing. The sensorium remained completely clear, and he was able to answer questions readily and correctly. Symptoms were maximal about 2 hr after their onset, at which time the pulse rate was 64 per minute (decreased from the subject’s normal resting rate of 70), and the respiratory rate was 18 per minute. During the entire course of poisoning, no miosis, excess lacrimation or salivation, or rales were observed. Definite improvement, including some increase in strength, appeared a little less than 3 hr after the onset of symptoms, and recovery was nearly complete 4 hr after onset.”

Finally, Baron cites a NIOSH study in which two workers exposed to airborne carbaryl for two workdays at a concentration of 50 mg/m^3 experienced no signs of intoxication.

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Branch and Jacqz (1986) described the toxic sequelae in a 75-year old man exposed accidentally, but over a prolonged period, to carbaryl. Symptomology ranged from acute to chronic. The basement of the man’s home was subjected to six monthly treatments with a 10% preparation of Sevin dust to combat fleas. (According to the report, this was inconsistent with the standard recommendation that a 2% preparation be used under these circumstances.) The air conditioner, which was located in the basement, dispersed the carbaryl throughout the house.

The subject developed influenza-like symptoms within 3 days of initial exposure, and headache, malaise, epigastric discomfort and muscle spasms on the fifth day. Progression of the symptoms, now including weight loss, occurred over the following month. An increase in symptom severity - severe spasms (at one point during the 6-month period requiring hospitalization), pressure headaches, rhinorrhea, tinnitus, vertigo, mild ataxia, muscle weakness and muscle fasciculations - was noted after the second monthly treatment. The subject became concerned that dementia might be developing.

The cause of the symptoms was not identified until 8 months of continued exposure, over which there was progressive symptomology. Blood studies, initiated following a first attempt to clean up the house at 8 months, revealed plasma cholinesterase levels at 64% of a “normal” value, confirming exposure to a cholinesterase inhibitor. RBC cholinesterase activity appeared normal.

The symptoms persisted or worsened despite two attempts to clean the house. Low abdominal discomfort led to the development of bilateral inguinal hernias, which were corrected following hospital admission at 10 months. Plasma cholinesterase levels returned to normal within two days of this hospitalization, accompanied by symptomatic improvement, though the surgeon was concerned for the apparent fragility of his tissues.

The subject moved to a motel following the surgery. His symptoms remained improved, though he experienced headache, nasal congestion and lacrimation upon home visits. An irregular pulse at one month post discharge led to readmission to the hospital with sinus bradycardia accompanied by multiple ventricular ectopic beats, low plasma cholinesterase and mild weakness. Once again, symptomology improved during the hospitalization. The subject then moved to a new home, experiencing a marked improvement of his symptoms, though his sleep pattern, which was accompanied by headache, tinnitius and confusion, remained altered during the following two years. A relocation to yet another home witnessed abatement of most symptoms. However, neuropathy (referred to as a "glove-and-stocking peripheral neuropathy") became more severe. This complication worsened over the following 15 months. Tomography revealed progressive dilation of the cerebral ventricles indicative of reduced cerebral function.

The authors state that the progressive, but non-specific, neurologic dysfunction that they described in this subject may be indicative of a wider clinical problem.

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Dickoff *et al.* (1987) described a case in which a 23-year old man purposely swallowed 100 ml of Ortho-Liquid-Sevin (27% carbaryl in water), equivalent to a dose of ~500 mg/kg body weight. The same individual had consumed an unknown quantity of boric acid on the same day and a "small amount" of dicumarol rat poison the day before. The observed effects were attributed to the very high carbaryl exposure (*i.e.*, comparable to the rodent LD_{50}). The following observations were recorded (this list is largely quoted from the manuscript):

(1) found comatose 3 hr post carbaryl ingestion;

(2) emergency room parameters, Day 0: coma, excessive salivation, miosis (1.5 mm pupils [nonreactive]), rhythmic asynchronous eyelid twitches / fasciculations, spontaneous roving eye movements, corneal reflexes present, flaccid tone, pulmonary edema, diarrhea, incontinence, 70 mm Hg systolic blood pressure, 100/min heart rate, 34°C body temperature (returned to normal by 12 hr), 7.13 arterial blood pH, 50 mm Hg P_{CO2}, 54 mm Hg P_{O2}, intubation for breathing and profuse bronchial secretion control, unresponsive to voice or pain, no spontaneous limb movement, tendon reflexes normal, ankle clonus but no plantar response, serum chemistries normal, brain CT normal, urine and blood toxicologic parameters normal, acute muscarinic toxicity resolved by 12 hr;

(3) emergency room parameters, Day 1: responsive to name, could blink, moved eyes and limbs, intubation discontinued, persistent dark brown heme negative urine, diarrhea for 48 hr;

(4) Day 2: followed commands and conversed, abdominal cramping;

(5) Day 3: prickling foot pains, progressing in 24 hr to legs and hands, whole blood ChE 4 U/ml (normal: 3-8 U/ml);

(6) Day 5: diffuse pain, leg paralysis, absent tendon reflexes, occasional rapid involuntary flexion of knees and hips, hand weakness, could not sit alone, glove and stocking sensory loss, pseudoathetotic [5] arm movements;

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[5] Athetosis: "a derangement marked by ceaseless occurrence of slow, sinuous, writhing movements, especially severe in the hands, and performed involuntarily" (Dorland's Illustrated Medical

(7) Day 6: proximal right leg movements, CSF contained 2200 RBC/mm^3, 20 WBC/mm^3, 65 mg% protein, normal conduction velocities with borderline amplitude of evoked compound muscle action potential in peroneal nerve, no voluntary motor units or only single unit recruitment patterns in distal leg muscles, normal sensory responses, no abductor digiti quinti response decrement after repetitive ulnar nerve stimulation, symmetrically diffuse ERG;

(8) Week 3: impaired finger strength, inability to stand, plantar responses were flexor, persistent tenderness to distal palpation, marked impairment to pin and vibration below the knees, absent position sense in toes and impaired in ankles (normal in fingers);

(9) Week 5: bilateral footdrop, no volitional motor units below knees, pin sensation absent in stocking distribution, toe position / vibration absent, diminished “CMAP” amplitudes in tested nerves, normal conduction velocities in arms with slight slowing in legs, evoked sensory nerve responses showed low amplitudes, increased insertional activity in EMG, muscle fibrillations and positive waves, periods of diffuse /symmetric slowing with EEG;

(10) Month 9: normal strength except for bilateral ankle / toe weakness, jerks elicited in triceps only, persistent loss of toe vibration / proprioception, pin and touch responses reduced to midcalf, normal EEG.

The authors claim that one day after ingestion there were no signs of cholinergic overactivity. They suspect that carbaryl induced a delayed polyneuropathy possibly similar to the delayed syndrome known to occur with organophosphate exposures. It is not known if binding to neurotoxic esterase or the subsequent “aging” reaction was involved in this case.

Dictionary, 26th Edition, 1985; W.B. Saunders Company; p. 134).

3. Laboratory animal studies

LD_{50}, LC_{50} and primary irritation data for carbaryl and for various end-product formulations containing carbaryl as the only active ingredient are listed in Tables III.2a and III.2b.

Table III-2a. The acute toxicity and primary irritation properties of technical grade carbaryl in multiple species

Species	Toxicity Category	LD_{50} or LC_{50}	References
Oral LD_{50}			
Rat, M	II	233-840 mg/kg	a-e
Rat, F	II	246-610 mg/kg	a-e
Rat, F	II	437.5 mg/kg	i
Mouse, M/F	II	108-650 mg/kg	d
Mouse, F	III	515 mg/kg	i
Rabbit (sex not reported)	III	710 mg/kg	a
Guinea pig (sex not reported)	II	280 mg/kg	a,d
Dog (sex not reported)	II	250-795 mg/kg	d
Cat (sex not reported)	II	125-250 mg/kg	d
Swine (sex not reported)	III	1500-2000 mg/kg	d
Deer (sex not reported)	II	200-400 mg/kg	d
Monkey (sex not reported)	III	>1000 mg/kg	d
Intraperitoneal LD_{50}			
Rat, M-adult	n/a	64 mg/kg	l
Rat, M-weanling (23 days)	n/a	48 mg/kg	l
Dermal LD_{50}			
Rat, M/F	III	>2000 - >5000 mg/kg	d
Rabbit, M/F	III	>2000 mg/kg	b,f
Inhalation LC_{50}			
Rat, M/F - 4 hours	III	0.873 mg/L	g
Rat, M/F - 4 hours	III	2.50 mg/L	h
Eye irritation			
Rabbit	IV		b
Dermal irritation			
Rabbit	IV		b
Dermal sensitization			
Guinea pig	negative		j,k

[a] Mellon Inst. (1957)
[b] Union Carbide (1983a-d)
[c] Union Carbide (1985)
[d] Cranmer (1986)
[e] Larson (1987d)
[f] Larson (1987a) - As the test article, Carbaryl 90DF, was "slightly moistened to make pasty", it is assumed that the moistening agent was water.
[g] Holbert (1989)
[h] Dudek (1985)
[i] Rybakova (1966)
[j] Larson (1987c)
[k] USEPA (2002a)
[l] Brodeur and DuBois (1963)

Table III-2b The acute oral toxicity of carbaryl formulations in the rat

Species	Tox. Categ.	LD_{50} or LC_{50}	References
Oral LD_{50}			
5% Sevin Dust / rat	III	4.49 g/kg (sex not stated)	a
7.5% Sevin Dust / rat	III	2.00 g/kg (sex not stated)	a
50% wettable powder / rat	II	0.23 g/kg (M)	b
13% emulsifiable. conc. / rat	II	0.71 g/kg (M)	b
Parid Bomb Plus (2.5% CL) / rat	III	>1.5 g/kg (M/F)	c
Sevin FR (40% CL) / rat	III	750 mg/kg (M); 527 mg/kg (F)	d
Sevin XLR (43% CL) / rat	III	642 mg/kg (M); 472 mg/kg (F)	e
Sevin 80 (80% CL) / rat	II	406 mg/kg (M); 203 mg/kg (F)	f
Sevin 50 MC (50% CL) / rat	III	1070 mg/kg (M); 406 mg/kg (F)	g
Sevin, 20% Bait / rat	III	3.25 g/kg (M/F)	h
CC 12152 (SX-1400) (13.5% CL) / rat	III	1.15 g/kg (M); 1.05 g/kg (F)	i
Sevin 10 Dust (10% CL) / rat	III	2.9 g/kg (M); 1.6 g/kg (F)	j
Sevin 4F (42.3% CL) / rat	III	945.2 mg/kg (M); 1031.3 mg/kg (F)	k
Sevin 4-Oil (47% CL) / rat	II	963.1 mg/kg (M); 473.3 mg/kg (F)	l
Sevin Brand XLR Plus (43.1% CL) / rat	III	486 mg/kg (M); 251 mg/kg (F)	m
Sevimol Brand 4 (40.5% CL) / rat	III	1180.9 mg/kg (M); 473.3 mg/kg (F)	n
Sevin Brand XLR Plus (44.3% CL) / rat	III	867 mg/kg (M); 575 mg/kg (F)	o
Adams Flea & Tick Dust II (12.5% CL) / rat	III	1853 mg/kg (M); 1718 mg/kg (F)	p
Sevin 4-Oil (47.3% CL) / rat	III	734.5 mg/kg (F)	q
Sevimol (40.3% CL) / rat	II	353.6 mg/kg (F)	r
Sevin Brand Granular Carbaryl Insecticide (6.3% CL) / rat	III	3310 mg/kg (M); 2330 mg/kg (F)	s
MS9-558 (13% CL) / rat	III	2230 mg/kg (M); 695 mg/kg (F)	t

[a] Myers and Homan (1978)
[b] Mellon Inst. (1957)
[c] Biosearch, Inc. (1980)
[d] Weatherhostz (1982)
[e] Myers (1983a)
[f] Myers (1983b)
[g] Myers (1985)
[h] Field (1980)
[i] Fukuda (1983)
[j] Duke (1982)
[k] Kuhn (1991a)
[l] Kuhn (1991b)
[m] Kuhn (1991c)
[n] Kuhn (1991d)
[o] Kuhn (1991e)
[p] Mitchell (1991)
[q] Kuhn (1992a)
[r] Kuhn (1992b)
[s] Myers (1987)
[t] Kuhn (1991f)

Moser (2007) investigated the effects of a single gavage dose of carbaryl on cholinesterase activity (brain and RBC) and motor activity in adult (92 days) and young (postnatal [pnd] days 11 and 17) male Long-Evans hooded rats. The doses were 0 (corn oil vehicle), 3, 7.5, 15 and 30 mg/kg body weight. ChE assays from tissue samples were performed 40 minutes after dosing, with special care taken to minimize carbaryl dissociation from the enzyme during the radiometric procedure. Motor activity, a measure of neurotoxicity, was gauged in the pnd17 rats only. This was done 15 minutes after dosing using a single 20-minute activity session conducted in a figure-eight chamber. The results of the pnd17 motor activity assays were compared to previously collected data in adult animals. The number of animals examined per dose was based on the expected variability of the ChE and neurotoxicity endpoints in young and adult rats. Thus for adults, 6 animals per dose were used, so that a statistically significant 10% change in enzyme activity could be detected. Eight animals per dose were used for the pnd11 animals due to the higher enzyme variability at the younger age. For the pnd17 animals, 10 animals per dose were tested because the neurotoxicity assays were known to be more variable than the ChE assays - thus a statistically significant 30% change in motor activity would be detected by this number of animals.

Neither deaths nor severe toxicity were noted during the very short time period of this study (40 minutes). Brain ChE from pnd11 animals was more sensitive to inhibition by carbaryl than the equivalent enzyme from pnd17 or adult animals. Thus for the pnd11 animals, activities at all doses were lower than controls by statistically significant margins, precluding assignment of a NOEL for brain ChE inhibition in this study (Table III-3). Statistically significant brain ChE inhibition in pnd17 and adult animals was noted at 7.5 mg/kg and above, though it is noted that activities were lower than controls at 3 mg/kg by non-statistically significant margins. LED_{10} (ED_{10}) values for brain ChE inhibition in pnd11, pnd17 and adult animals, calculated by the study statistician (W. Setzer) using an exponential algorithm, were 1.14 (1.46), 2.37 (3.00) and 2.03 (2.63) mg/kg, respectively. Parallel LED_{10} (ED_{10}) values for RBC ChE inhibition in pnd11, pnd17 and adult animals were 0.78 (1.11), 1.05 (1.41) and 0.73 (0.96) mg/kg, respectively. The pnd11 LED_{10} value for brain ChE inhibition, 1.14 mg/kg [rounded to 1.1 mg/kg], was used in USEPA's Reregistration Eligibility Decision document to estimate acute risk from carbaryl exposure (USEPA, 2007a).

Statistically significant decreases in motor activity were noted at the high dose only in the pnd17 animals. The motor activity data for adult animals from a previous study suggested that adults were somewhat more sensitive to carbaryl than pnd17 animals with respect to this parameter.

As this was not a FIFRA-guideline study, it was considered to be supplemental.

Table III-3. Brain and RBC cholinesterase activities, motor activities and ED_{10} and LED_{10} values following a single gavage dose in male Long-Evans rats (Moser, 2007)

	Carbaryl, mg/kg						
	0	**3**	**7.5**	**15**	**30**	**LED_{10} [a]**	**ED_{10} [a]**
Brain ChE [b]							
Pnd 11 (n=8)	3.70±0.32 100±9	3.00±0.40 ** 80±11	2.38±0.29 ** 64±8	1.89±0.30 ** 51±8	1.60±0.27 ** 43±7	1.14	1.46
Pnd 17 (n=10) [e]	4.99±0.33 100±7	4.55±0.44 91±9	3.77±0.52 ** 76±11	3.26±0.31 ** 65±6	2.64±0.48 ** 53±10	2.37	3.00
Adult (n=6)	6.38±0.58 100±9	5.86±0.67 92±11	4.76±0.27 ** 75±4	4.01±0.60 ** 63±9	3.25±0.29 ** 51±5	2.03	2.63
RBC ChE [c]							
Pnd 11 (n=8)	0.64±0.15 100±23	0.50±0.19 78±29	0.34±0.10 ** 53±16	0.23±0.06 ** 37±10	0.17±0.05 ** 27±7	0.78	1.11
Pnd 17 (n=10)	0.69±0.14 100±20	0.57±0.14 84±21	0.37±0.11 ** 55±16	0.31±0.06 ** 45±8	0.20±0.09 ** 29±13	1.05	1.41
Adult (n=6)	0.61±0.05 100±8	0.39±0.05 ** 64±8	0.30±0.03 ** 50±5	0.21±0.05 ** 34±8	0.14±0.08 ** 24±13	0.73	0.96
Motor activity [d]							
Pnd 17 (n=10)	114.6±37.0 100±32	94.6±33.7 83±30	113.4±40.1 99±35	88.0±30.5 77±27	56.6±35.3 ** 49±31	nd	nd

Abbreviation: nd, not determined

**, $p \leq 0.01$, Dunnett's parametric t test (performed by DPR)

[a] LED_{10} and ED_{10} values, which are expressed as mg/kg, were calculated by the study author using an exponential algorithm.

[b] Brain cholinesterase activities are expressed both in units of µm ACh hydrolyzed / min / mg protein and in percent of concurrent controls.
[c] RBC cholinesterase activities are expressed both in units of µm ACh hydrolyzed / min / ml RBCs and in percent of concurrent controls.
[d] Motor activities are expressed as total counts per 20-minute test period.
[e] The only exceptions with regard to the n value for pnd 17 rats were the 0 and 15 mg/kg brain ChE dose groups, for which n=9.

In a rangefinding acute toxicity study, Brooks and Broxup (1995a) administered carbaryl (99.1%) by gavage to 2 rats/sex/dose (Sprague Dawley) at 10, 50, 100, 250, 500 or 1000 mg/kg (no control group). The vehicle was 0.5% (w/v) carboxymethylcellulose / 0.1% Tween 80 (10 ml/kg). Dosing was followed by a 3-day observation period for clinical signs and mortality. Body weights were recorded on days 0, 1 and 3. Necropsies were not performed. Physical exams were performed pre-dose, at 0.5, 1, 2, 4 and 8 hr post-dose, and on days 1, 2 and 3.

At 1000 mg/kg all animals were dead within 24 hr. At 500 mg/kg, 1/2 males and 2/2 females were dead within 24 hr. All animals survived at 250 mg/kg. Within 30 minutes in both sexes, all rats at $\geq$50 mg/kg exhibited slight to severe salivation and tremors of head, body and/or limbs. Lacrimation, periorbital staining, urogenital staining, decreased activity, decreased respiration rate, abnormal breathing sounds and weakness were seen in some or all groups at $\geq$50 mg/kg. With the exception of staining, decreased activity and weakness, many of the signs were no longer observed 1 day after dosing. Weight losses were observed at all doses >10 mg/kg.

A conditional NOEL of 10 mg/kg was established, based on clinical signs at 50 mg/kg and above. However, the low number of animals and limited observational time (3 days) diminished the reliability and regulatory importance of this value. This study was considered to be supplemental.

In a follow-up study designed to determine the time to peak effects after a single oral dose, Brooks and Broxup (1995b) treated Sprague-Dawley rats by gavage with technical grade carbaryl (99.1%). The doses were 0, 10, 50 or 125 mg/kg. As before, the vehicle was 0.5% (w/v) carboxymethylcellulose / 0.1% Tween 80 (10 ml/kg). Three animals/sex/dose were included in the behavioral phase, which consisted of an abbreviated functional observational battery (locomotor activity, gait, tremor, twitches, convulsions, behavior, respiratory rate, lacrimation, salivation, staining and diarrhea) conducted at 0.5, 1, 2, 4, 8 and 24 hr (termination). Whole blood, plasma and brain cholinesterase determinations were done at termination for these animals. An additional 15 animals/sex/dose were included in the cholinesterase phase (whole blood, plasma and brain enzymes), with 3/sex/dose terminated at 0.5, 1, 2, 4 or 8 hr. RBC and plasma cholinesterase levels were also determined pre-dose.

Except for one 10 mg/kg male exhibiting muzzle/urogenital staining at 0.5 hr, FOB changes and/or clinical signs were seen only at 50 and 125 mg/kg. FOB changes at all dose levels, including tremors and autonomic signs, exhibited a time to peak effect in the 0.5-1 hr range, generally lessening after that time. A behavioral NOEL could not be assigned.

Brain cholinesterase activities showed marked inhibition at 0.5 hr (activities were 46%**, 23%** and 18%** of concurrent controls at increasing doses in males, 54%**, 24%** and 22%** of controls in females) and 1 hr (males 68%**, 25%** and 22%** of controls; females: 64%**, 23%** and 16%**; $p<0.01$, Dunnett's test), declining steadily thereafter, though inhibition was still present after 24 hr at 125 mg/kg in both sexes at the high dose (77% and 65%** of controls). A similar pattern was evident for whole blood cholinesterase activities, though the extent of the inhibition was somewhat less than for brain. Plasma cholinesterase activities were markedly inhibited at 0.5 and 1 hr (males, 0.5 hr: 64%*, 24%** and 19%** of controls; females, 0.5 hr: 62%, 29%* and 31%*; males, 1 hr: 68%*, 27%** and 17%**; females, 1 hr: 71%*, 25%** and 11%). Substantial recovery had occurred by 24 hr except at the high dose where male and female activities were 59%* and 46%** of controls.

Based on the clear inhibition of all cholinesterases (including brain cholinesterase) at 10 mg/kg, this dose was designated as a LOEL for this study. This study was deemed supplemental.

A third study in this series was designed determine the time course of cholinesterase inhibition in rats after acute oral exposure to carbaryl (Brooks and Broxup [1995c]). Carbaryl (99.1%) was given by oral gavage to Sprague-Dawley rats at doses of 0, 10, 30 or 90 mg/kg. The vehicle was 0.5% (w/v) carboxymethylcellulose / 0.1% Tween 80 (10 ml/kg). There were 24 rats/sex/dose, with 6/sex/dose sacrificed at 1, 8, 24 or 48 hr after dosing. Blood, brain and several brain regions were processed for determination of cholinesterase activity. Whole blood and plasma activities were measured and RBC activity was calculated from these measurements after determining hematocrits. “Whole” brain enzyme measurements were conducted using the left hemisphere. Brain regional measurements (frontal cortex, hippocampus, cerebellum and caudate/putamen) came from the right hemisphere. Clinical signs were also monitored.

No clinical signs were reported at 10 mg/kg. At 30 and 90 mg/kg signs included tremors (slight at 30, moderate to severe at 90 mg/kg), salivation, staining of fur and wetness in various areas on the day of treatment, with an occasional clinical siting at 90 mg/kg up to 2 days (study termination).

Cholinesterase activities were statistically lower in all samples from the 30 and 90 mg/kg groups and in most samples at 10 mg/kg. By 8 hr all samples at 10 mg/kg were comparable to controls. By 24 hr all samples at 30 mg/kg were comparable to controls. By 48 hr, all samples at all doses were comparable to controls. Brain regional assays did not show qualitative differences. Based on the inhibition of all cholinesterases at 1 hr at 10 mg/kg (including the various brain cholinesterases, which showed activities of 57%-73% of concurrent controls at that time), this dose was designated as a LOEL for this study.

This study was deemed supplemental.

A fourth study in the series was designed to study the behavioral and possible neuromorphologic effects of acute gavage exposure to carbaryl in Sprague-Dawley rats (Brooks *et al.* [1995]). Carbaryl (99.1%) was given in a single oral dose to 12 rats/sex/dose at 0, 10, 50 or 125 mg/kg using 0.5% (w/v) carboxymethylcellulose / 0.1% Tween 80 (10 ml/kg) as the vehicle. Examinations for mortality and clinical signs were performed daily. Body weights and food intake were assessed weekly. Functional observational batteries (FOB) and motor activity assessments were performed both prior to and after dosing on day 0 (0.5 hr after for the FOB and 50-90 min for the motor activity assessment, to correlate with the time of peak effect documented in Brooks and Broxup [1995b]), and on days 7 and 14. At study termination on day 15, 6/sex/group were processed for neuropathology examinations. The remaining 6/sex/group were necropsied.

There were no deaths. Clinical signs, noted in females at 50 mg/kg and in both sexes at 125 mg/kg, were dominated by observations of fur staining and ocular signs. Mid and high dose males exhibited reduced body weight gains during the first week, but appeared to compensate during the second week (mean male weight gains in grams, week 1, at ascending doses: 41.8, 40.7, 32.4*, 13.0**; week 2: 35.6, 37.5, 34.1, 41.3; *, **p<0.05, 0.01). High dose females exhibited reduced body weight gains during the first week, but also appeared to compensate during the second week (mean female weight gains in grams, week 1: 16.5, 16.5, 13.0, 9.6; week 2: 13.3, 15.2, 13.5, 22.9*; *p<0.05). Food intake was decreased during week 1 (mean male intake in grams/rat at ascending doses, week 1: 183.9, 203.7, 181.5, 148.4**; week 2: 204.8, 214.3, 195.9, 188.1; mean female intake, week 1: 132.5, 138.9, 127.2, 109.3**; week 2: 135.6, 145.6, 137.5, 141.7; **, p<0.01).

FOB analysis revealed effects in both sexes at 50 and 125 mg/kg on day 0. In many instances dose responsiveness was evident with respect to severity and incidence, with most observations achieving statistical significance. These included ⇑ incidence of salivation and / or

wet muzzle, [incidence of tremors, ataxic gait / overall gait incapacity, \ locomotor activity, arousal and # of rears, [positional passivity, \ extensor thrust, tail and toe pinch responses, impaired visual placing response, \ urination and defecation in males, \ vocalization upon cage removal in females, [auricular startle response (high dose), [incidence of males lying on ventral surface (high dose), \ fore and hind grip strength (high dose), [hindlimb splay (males, high dose), and \ body temperature. These effects had largely abated by days 7 and 14.

There were marked decreases in motor activity counts (>75%) over 60 minutes in both sexes at 50 and 125 mg/kg on day 0 (Table III-4). Motor activity counts at 10 mg/kg were also slightly lower than controls (males: 177.2* vs. 221.7; females: 314.3* vs. 393.8; $p<0.05$). However, the study authors discounted the possibility that the 10 mg/kg observation was treatment induced, citing the predose variability, the lack of similar findings in the concurrent FOB, and the fact that the values were within the historical control range for the laboratory (however, only the male historical control range of 130-361 counts was presented). On the other hand, it is likely that brain cholinesterase inhibition was present at this dose - the same authors had observed marked inhibition at 10 mg/kg at 0.5 hr (the time to peak effect) in one of the previous studies in this series (Brooks and Broxup [1995b]). In addition, (1) the effect was noted in both sexes, (2) the direction of the motor activity change (*i.e.*, a lowered activity in the presence of 10 mg/kg carbaryl) was consistent with the motor activity and FOB observations at the higher doses, and (3) the pattern of motor activity observations through the hour-long assay in both sexes, recorded in 10-minute intervals, indicated a depressive effect of carbaryl early in the hour (when the animals were much more active). The extent of the carbaryl effect decreased later in the hour, when the activity of all of the animals (controls and dosed) had declined precipitously. Thus the decrease in motor activity counts at 10 mg/kg was considered toxicologically significant for the purposes of risk assessment.

Necropsies and histopathologic analyses did not yield carbaryl-related effects. This was also true for the brain weight and size determinations performed at study termination.

A NOEL was not determined for this study. The LOEL was set at 10 mg/kg based on a reduction in motor activity counts at that dose. The study was considered acceptable by FIFRA guidelines.

Table III-4. The impact of carbaryl exposure (acute, oral gavage) on motor activity [a] in Sprague-Dawley rats; 1-hr mean data (Brooks *et al.*, [1995])

	Males				Females			
	0 mg/kg	**10 mg/kg**	**50 mg/kg**	**125 mg/kg**	**0 mg/kg**	**10 mg/kg**	**50 mg/kg**	**125 mg/kg**
Pre-study	255.3±87.6	303.2±92.8	294.1±104.8	252.0±85.3	298.8±112.8	272.4±66.6	316.9±140.3	250.5±97.9
Day 0	221.7±51.3	177.2±59***	53.7±32.4***	35.8±23.7***	393.8±127.6	314.3±101.6[#]	36.9±31.4[###]	47.8±65.3[###]
Day 7	217.9±57.0	281.3±57.7	322.8±139.0	193.3±88.1	425.9±107.2	391.8±143.6	503.8±161.7	298.4±138.4
Day 14	246.3±80.1	293.6±98.7	347.7±153.6	227.5±94.5	452.9±132.2	406.3±115.5	451.1±131.4	362.3±158.9

***: $p<0.001$, t-test, linear constructed variable

[#], [###]: $p<0.05$, 0.001, Wilcoxon, linear constructed variable

[a] Motor activity was determined in a “Figure 8 enclosure” based on interruption of a light beam. The 1-hr sessions were recorded by a microcomputer in 30 successive 2-min intervals. The mean number of counts/hr ± the standard deviation is expressed in this table.

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Beyrouty (1992a) examined the time to peak effect in Sprague-Dawley male rats, 2/dose, from an acute gavage dose of carbaryl (98% purity). Doses were 0 (10 ml/kg aqueous 0.5% carboxymethylcellulose / 0.1% Tween 80), 25, 80 or 250 mg/kg. There was no analysis of the test material. Animals were observed for a 7-day period, including body weight measurements. An abbreviated FOB was conducted at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hr post dose.

There were no deaths, despite the fact that the high dose approached or exceeded the LD_{50} for this compound. High dose animals showed decreased arousal and locomotor activity, with the largest effect at 1-1.5 hr. They also exhibited incapacitated gait, tremors, salivation, lacrimation (beginning @ 4 hr), urinary staining (beginning @ 4 hr), and reduced respiration. Mid dose animals showed decreased locomotor activity (greatest effect, 0.5-3 hr), decreased arousal (greatest effect, 0.5-1 hr), incapacitated gait, tremors, salivation and reduced respiration. Effects on body weight were seen at 80 and 250 mg/kg. No clear effects were detected at the low dose. The estimated time to peak effect was 0.5-1.5 hr. The NOEL was set at 25 mg/kg. This was a non-FIFRA-guideline study.

In a more extensive study, Beyrouty (1992b) examined the effects of carbaryl after acute oral administration to Sprague-Dawley rats. Twelve males per dose were treated with a single gavage dose at 0 (10 ml/kg aqueous 0.5% carboxymethylcellulose / 0.1% Tween 80), 12.5, 40 or 125 mg/kg carbaryl (98% purity). There was no analysis of the test material. Twice daily observations for clinical signs and mortality were conducted. Body weights were determined weekly. FOBs and motor activity evaluations were carried out pretest and on days 0 (day of treatment), 1, 7 and 14. Histopathologic exams were conducted on brain and abnormal tissues.

There were no deaths during the 2-week course of the study. A 27 g loss of body weight at 125 mg/kg between days 0 and 1 resulted in significantly lower body weights on days 1 and 7 (9.5% and 6%, respectively; $p<0.01$). Body weight effects were not apparent at the other doses. FOB analysis on day 0 showed the following effects at 125 mg/kg: tremors; gait incapacity; salivation; miosis; decreased locomotor activity, arousal and defecation; abnormal responses to sensory tests and others. Day 0 FOB effects at 40 mg/kg included tremors; salivation; and decreased locomotor activity, arousal, toe/tail pinch and defecation. Defecation was also statistically reduced at 12.5 mg/kg on day 0 (# of fecal “boli” during a 2-min period in the day 0 FOB: 1.9±1.4, 0.8±1.0*, 0.8±0.7*, 0.0±0.0***; *$p<0.05$; ***$p<0.001$). However, these data were very hard to interpret in view of the short observation period. Forelimb and hindlimb grip strength were reduced on day 0 at 125 mg/kg, with hindlimb grip strength also reduced at 40 mg/kg. Foot splay was significantly increased in both dose groups on Day 0. Body temperature was reduced on day 0 for all dose groups (°C): 38.0±0.33, 37.3±0.99*, 34.9±0.53** and 34.3±0.78** (*$p<0.05$; **$p<0.01$). There was, however, concern that the control body temperatures were inappropriately high, casting doubt on the apparent temperature lowering response at 12.5 mg/kg. Group mean total activity counts were lower at 40 and 125 mg/kg (209, 207, 43.3*** and 23.7***; ***$p<0.001$). Total activity counts were still statistically depressed on day 1 at the high dose, though no significant differences remained by days 7 and 14.

Based on the FOB and decreased body temperature findings at 40 and 125 mg/kg, the LOEL was set at 40 mg/kg. The apparent effects on defecation and body temperature at 12.5 mg/kg were not considered strong enough to establish a LOEL, though they were certainly indicators that adverse effects could be detected at that dose in other studies. This study was considered supplemental.

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In an open literature study, Moser *et al.* (1988) used the functional observational battery (FOB) to discern probable neurotoxic responses in Long-Evans hooded rats, 10/sex/dose, to a single

intraperitoneal dose of carbaryl . The carbaryl doses were 0 (vehicle control: 5% ethanol-5% Emulphor in saline), 3, 10 and 30 mg/kg. The effects of chlordimeform were also examined, though those results will not be summarized here. The FOB tests were run prior to dosing and at 0.5, 3, 24 and 48 hours post dose. The following parameters were examined: posture, palpebral closure, presence or absence of writhing, circling, biting or vocalizations, ease of removal / handling, observable signs (exopthalmus, crustiness around the eyes, piloerection, bite marks on tail or paws, missing toenails, body tone and emaciation), “cart top” measurements (latency to first step, number of rears [supported and unsupported], grooming episodes, gait characteristics, arousal level, number of fecal boluses and urine pools), reflex testing (responses to approach of a pencil, a touch to the rump, finger snap, tail pinch), pupil contraction to light, extensor thrust, limb rotation, degree of catalepsy, righting reflex, grip strength, foot splay, body weight and rectal temperature. The entire exam required 6-8 minutes per rat.

Effects noted at both 10 and 30 mg/kg included the following (in both sexes unless otherwise noted): decreased rearing, decreased arousal, home cage posture alteration, decreased removal difficulty (males only), convulsions, increased urination (females at high dose only), home cage palpebral closure (females at high dose only), pupil response, righting reflex, decreased approach response (females at high dose only), decreased finger snap response (males at high dose only), decreased touch response, decreased tail pinch response, chewing motions, decreased rectal temperature and decreased body weight (females at high dose only). Effects noted at 30 mg/kg only included increased latency to first step (males only), abnormal fur appearance, decreased defecation, salivation, piloerection (males only), affected gait, decreased forelimb grip strength (females only), palpebral closure at handling (males only), generalized tremors and catalepsy. For those parameters for which time data were reported, the most severe responses were noted at 0.5 and 3 hr post dose. There was limited evidence for a slight increase in unsupported rears in males at 3 mg/kg, though dose dependence was not in evidence. In the absence of other signs at that dose, this particular observation was considered inadequate to determine a LOEL.

The acute NOEL was set at an intraperitoneal dose of 3 mg/kg, based on a plethora of FOB observations at 10 mg/kg/day.

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Weinberg (2008) administered aerosolized carbaryl technical (99.8% purity) through nose-only devices to two separate cohorts of Crl:CD (SD) rats. Exposure was for a single 3-hr period using 5 animals/sex/dose. In the first cohort, the animals were exposed at 0, 63, 121 or 247 mg/m^3 (gravimetric analysis). The respective mean mass median aerodynamic diameters (geometric standard deviations) for the exposed groups were 1.6 (2.15), 1.6 (2.18) and 1.7 (2.23) µm, respectively. In the second cohort, males were exposed to 0, 12, 29 or 55 mg/m^3, with MMAD (GSD) values of 2.1 (2.25), 2.0 (2.19) and 2.0 (2.22) µm. The females in this cohort were exposed to 0, 10, 27 or 65 mg/m^3, with MMAD (GSD) values of 2.1 (1.92), 2.1 (2.28) and 2.0 (2.22) µm, respectively. RBC and brain cholinesterase activities were determined immediately upon termination of exposure. According to the text of the study, precautions were taken to minimize dissociation of carbaryl from the enzyme during the assay. Gross necropsies were also performed and brain weights determined at that time.

All animals survived the exposure period. Necropsies and brain weight determinations were unremarkable. RBC and brain cholinesterase activities in the first cohort were reduced in a statistically significant, dose-dependent manner for both sexes in all exposure groups ($p<0.01$). Significant reductions were also noted at the mid and high doses in the second cohort (dose responsiveness was not evident in the case of the RBC enzyme). Reductions at the low doses

did not achieve statistical significance, though they were suggestive of effects. A LOEL of 10 mg/m^3 (1.2 mg/kg for the 3-hr exposure using the default breathing rate of 0.96 m^3/kg) was assigned based on the inhibition of brain cholinesterase in females at the low dose in cohort #2. Benchmark dose analysis of these data using the power algorithm (power unrestriced) resulted in LED_{10} (ED_{10}) values of 5.51 (13.29) mg/m^3, equivalent to 0.66 (1.59) mg/kg (Appendix V).

This study was considered to be supplemental, as it was not performed according to a FIFRA guideline protocol.

Table III-x. RBC and brain cholinesterase activities after a 3-hr acute inhalation exposure to carbaryl in rats (Weinberg, 2008)

Cohort 1:

	Carbaryl (mg/m^3) - males				Carbaryl (mg/m^3) - females			
	0	**63**	**121**	**247**	**0**	**63**	**121**	**247**
RBC, U/L **% of control**	3708±856	2317±182** 62.5%	1625±35** 43.8%	1040±484** 28.0%	4067±638	1551±620** 38.1%	955±446** 23.5%	831±460** 20.4%
Brain, U/L **% of control**	47391±1293	36116±1298** 76.2%	32512±3054** 68.6%	23302±4390** 49.2%	45767±2342	31991±4565** 69.9%	23563±4773** 51.5%	19861±2462** 43.4%

**p<0.01

Cohort 2:

	Carbaryl (mg/m^3) - males				Carbaryl (mg/m^3) - females			
	0	**12**	**29**	**55**	**0**	**10**	**27**	**65**
RBC, U/L **% of control**	4212±616	3765±567 89.4%	2931±402** 69.6%	3463±1980 82.2%	4508±530	4296±633 95.3%	3282±216** 72.8%	3236±369** 71.8%
Brain, U/L **% of control**	49920±2400	48515±2228 97.2%	41771±1295** 83.7%	40133±1980** 80.4%	51181±2414	47776±2966 93.3%	42833±1398** 83.7%	40506±1533** 79.1%

**p<0.01

C. SUBCHRONIC TOXICITY (including NEUROTOXICITY)

1. Overview

Three subchronic oral studies, in mice, rats and dogs, were available for analysis. As none of these studies was conducted according to FIFRA guidelines, each was considered supplemental. Neither the mouse or dog studies showed clear adverse effects. However, neurotoxic effects were observed in the rat with respect to maze performance, EEG readings and brain cholinesterase activities. As the changes in maze performance were manifested soon after the advent of dietary exposure, they were consistent with an acute effect. Three 4-week repeat dose dermal studies using 99.49%, 44.82% and 80.07% formulations were also conducted. Carbaryl inhibited both RBC and brain cholinesterases at a LOEL dose of 50 mg/kg/day (99.49% and 80.07% formulations). Except for possible weight decrements and local irritation noted with the 99.49% formulation, there were no other effects.

Subchronic NOELs and LOELs are summarized in Table III-8.

2. Laboratory animal studies

a. Oral exposure

Mice. Dange (1998) administered carbaryl (purity, 98.4%) in the diet to TSG p53 wild type male mice for at least 28 days. Diets of 0, 160, 1000, 2000, 4000 or 8000 ppm were fed to 10 mice per dose group in two different studies (0, 160, 1000 and 8000 ppm in one study, 0, 2000 and 4000 ppm in the other). These corresponded to mean compound consumption levels of 0, 35.7, 222.0, 424.4, 935.6 and 2107.3 mg/kg/day. All mice were necropsied on days 29 or 30. Bodyweight, mortality, clinical signs and organ weights were determined, though histopathology was not performed.

Neither deaths nor clinical signs were noted during the study. The 8000 ppm mice lost ~14% of their initial body weight during the first study week (23.26 g at the outset *vs.* 20.16** grams after week 1 for the high dose animals; 22.84 g *vs.* 23.77 g for the controls; **p<0.01). These animals did not recover their bodyweights by the end of the study (21.63** g *vs.* 24.89 g in controls after week 4). At 4000 ppm, the mice also sustained bodyweight losses during the first week (21.54 g at the outset *vs.* 21.19** g after week 1 for the 4000 ppm animals; 21.63 g *vs.* 23.16 g for the controls). By study termination the weight differences between the controls and 4000 ppm animals amounted to ~6%, though the differences were not statistically significant. Food consumption was statistically increased at 8000 ppm during the second week (6.16** g/day *vs.* 4.97 g/day in controls) and at 4000 ppm during the first week (5.82* g/day *vs.* 4.97 g/day in controls). The relative liver weights (compared to total bodyweight) were 6%*, 12%* and 16%* higher than controls at 2000, 4000 and 8000 ppm. There were no discernible effects on absolute liver weight, however. Relative kidney weights were also slightly higher at those doses, though statistical significance was not achieved. Necropsies did not reveal treatment-related abnormalities.

A conditional NOEL was set at 1000 ppm (222.0 mg/kg/day) based on the relative liver weight changes at 2000 ppm (424.4 mg/kg/day). However, since histopathology was not done, it is difficult to gauge the toxicologic significance of this change.

This study was considered to be supplemental.

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Rats. Desi *et al.* (1974) examined the neurotoxicologic effects in male Wistar rats (R strain) of daily dietary exposure to carbaryl. The exposure period was up to 50 days. Another carbamate, Arprocarb, was also tested, though those data will not be summarized here. The stated aim of the study was to provide information on (1) whether these compounds can be used safely over extended periods and (2) what the relationship is between cholinesterase inhibition and the observed neurotoxicity.

Rats received carbaryl (Sevin 85 WP) through the feed, which was administered at 10 g of feed/day/100 g body weight in order to ensure precise dosing. The final carbaryl doses were 10 and 20 mg active ingredient/kg/day. Animals were evaluated by means of: (1) T-maze experiments designed to determine the time to reach a goal and number of errors committed in that process (Test #1: 8 rats/group, 50-day test, to see how carbaryl affects the rate that rats learn how to negotiate the maze; Test #2: rats previously trained over a 15-day period to negotiate the maze were subjected to a 50-day test, 8 rats/group, to see how the pesticide affected the performance of the pre-learned task.); (2) EEG exams, which employed two frontooccipital electrodes on unrestrained animals after the 50-day period; (3) cholinesterase determinations on blood and brain parts (cortical gray matter, white matter, brain stem, cerebellum), also performed after the 50-day period, with special precautions taken to prevent dissociation of carbaryl from the enzyme.

During the first 2 weeks, both dose groups performed notably better in the mazes than

controls, achieving the goal 10-12 seconds faster at day 11 ($p<0.05$, for both groups). However, by day 25, there were no clear differences between treated animals and controls, a situation that was maintained through the end of the study at day 50. A similar observation was made for error frequency - while significantly fewer errors were made in both dose groups on day 11 ($p<0.001$), all groups (including controls) declined to a minimum error frequency around day 22-24, followed by a general increase in error frequency in all groups after that point with no clear difference between treated animals and controls.

If the animals were first trained in maze running for 15 days before the advent of treatment, maze performance continued to improve somewhat in controls (maze time at the end of training = 7.9 sec; maze time at 50 days = ~5 sec). Low dose animals may have continued to improve at a slightly higher rate than controls, though by 15 days feeding, controls and low dose animals could not be distinguished, and by 50 days, low dose animals negotiated the maze with statistically higher times than controls (~6 sec., *vs.* ~5 sec., $p<0.02$). On the other hand, high dose animals ceased the improvement with the advent of treatment. Their running times at days 21 and 50, were statistically higher than controls ($p<0.05$ and $p<0.02$, respectively). Error frequencies were statistically higher in both groups following the advent of treatment.

Carbaryl increased the frequency of basal brain electrical activity in both dose groups. This was particularly true of the γ (theta) wave frequency, which was statistically increased over controls at both doses ($p<0.01$) and the β2 wave frequency, which was statistically increased over controls at the high dose ($p<0.01$). Exposure to rhythmic light loading at 1.5, 5 or 11 Hz did not change the EEG characteristics. However, “markedly accelerated electrical activity” was seen in carbaryl-exposed animals at a stimulation rate of 18 Hz (though the data were not provided in the report).

Animals exposed to 20 mg/kg/day carbaryl for 50 days registered statistically lower cholinesterase activities in all brain regions examined. Thus cortex, white matter, brain stem and cerebellum exhibited 60.5%, 85.8%, 56.3% and 60.9% of control activities. Animals exposed to 10 mg/kg/day did not evidence statistically significant changes (though again, the data were not provided in the report). Plasma and RBC cholinesterase activities in treated animals were similar to controls. Dose-dependent, statistically significant increases in protein content were seen in all four brain regions.

The authors ascribed the increased learning ability detected in the first 2 weeks of maze testing to “enhanced irritability” of the central nervous system. Their conviction that the CNS was the main site of action was strengthened by the observation that “the animals were able to move quickly even during [the] second period” (*i.e.*, the period of decreased maze function). This was supported by the evidence that EEGs and brain cholinesterase activities were also altered by carbaryl exposure.

A LOEL of 10 mg/kg/day was established in this study based on changes in maze function, EEG characteristics and brain cholinesterase activities at that dose. The rapidity in which maze performance changed as a function of exposure suggested an acute basis for the effect. Such could not be said for the EEG and enzyme observations, which were made only after 50 days of daily exposure.

This study was considered to be supplemental.

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Dogs. Hamada (1991) studied the effects of carbaryl (purity, 99.3%), administered to beagle dogs for 5 weeks by the dietary route. There were 6 dogs/sex/dose. Doses were 0, 20, 45 and 125 ppm, corresponding to average systemic doses of 0, 0.59, 1.43 and 3.83 mg/kg/day in males and 0, 0.64, 1.54 and 4.11 mg/kg/day in females.

There were neither deaths nor clinical signs throughout the study. Body weights, food

consumption, ophthalmoscopy, RBC cholinesterase activities (measurements done on days -11, -8, -5, 14 and 32), brain cholinesterase activities (measurements on days 37-39) and gross pathology appeared unaffected by exposure. Statistically significant depressions of plasma cholinesterase activities (measurements on days -11, -8, -5, 14 and 32) were detected in day 14 males at the low and high doses (enzyme activities at increasing doses, in µM/ml, day 14: 8.9, 7.3*, 8.1, 6.9*; *$p \leq 0.05$). As there was no clear dose responsiveness, no sign of an effect in females, no statistically significant effects at day 32, and the "inhibition" characteristics were roughly shared by the same animals when measured on three separate occasions *before* the commencement of dosing (*eg.*, plasma ChE activities on day -8 were 9.1, 7.7, 8.6 and 8.3 µM/ml), the depressions on day 14 were not sufficiently clear to be considered a definite function of carbaryl exposure. Even so, the possibility of inhibition, particularly at the high dose, was not definitively excluded.

As there were no adverse effects noted, the subchronic NOEL was set at >125 ppm (M: 3.83 mg/kg/day; F: 4.11 mg/kg/day). Due to the short length of treatment, the limited parameters measured, the lack of histopathologic exams and the poor dose selection (*i.e.*, too low), this study was considered to be supplemental.

b. Dermal exposure

Rats. Austin (2002a) examined RBC and brain cholinesterase activities in Sprague-Dawley rats, as well as local and systemic signs, during 4 weeks of daily dermal exposure (6-7 hr/day, 5 days/wk) to carbaryl (99.49%). The test material, a slightly pink powder, was applied under gauze to moistened skin (~10% of the body surface area) at doses of 0, 20, 50 or 100 mg/kg/day. There were 10 animals/sex/group. They were observed twice daily for mortality and moribundity, while weekly observations (including on the first day of treatment) were made for clinical signs and dermal irritation. RBC cholinesterase activities were measured before the daily application on days -4, 1, 8, 15 and 22, and within 1 hr after dose removal on days 5, 12, 19 and 26. Brain cholinesterase was determined in the right half of the brain following sacrifice on day 26.

All animals survived the treatment. There were no behavioral or morphologic signs attributed to carbaryl exposure. Mean body weight gain for high dose males was statistically lower than controls for the day 5-12 period (weight gains at ascending doses, males, day 5-12: 33, 35, 34, 24* g; *$p \leq 0.05$). Decreased (though non-statistically significant) mean body weight gains in high dose animals were also noted over the time periods on either side of the day 5-12 period (*i.e.*, days -3-5 and 12-19). Whether or not the statistically increased weight gain in mid dose males for the day 19-26 period (19, 20, 26*, 23 g; *$p \leq 0.05$) was treatment related was not clear, though the lack of dose responsiveness is noted. Dermal irritation observations revealed a slight atonia (impairment of elasticity) in 1/10 and 4/10 high dose males and females, respectively.

RBC cholinesterase activities at 50 and 100 mg/kg/day were lower than parallel controls by statistically significant amounts in those samples taken within an hour of test article removal on days 5 (♂ & ♀) and 12 (♂ @ 100 mg/kg/day only, ♀ @ 50 & 100 mg/kg/day). On day 19, statistically significant inhibition was registered at 100 mg/kg/day in females only, while on day 26 no inhibition was noted in either sex (Table III-5). Regardless of sampling day, inhibitory effects noted after a 6-7 hr dosing period may be more a function of single, rather than multiple, exposures.

RBC cholinesterase inhibition was less apparent in those samples taken before the daily dosing, with statistically significant effects noted only in high dose males on days 8 and 22. No inhibition was noted in females. Inhibition in the pre-daily dose samples, when it was detected, might represent a effect of multiple dosing rather than an acute effect.

Brain cholinesterase activities measured on day 26 were 15% lower than controls in males at 50 and 100 mg/kg/day and 24% lower than controls in females at 100 mg/kg/day ($p<0.05$). It was not clear if the brain effect was due to a single or a multiple exposure, since samples were taken only after a 6-7 hr dermal exposure period on day 26.

The systemic NOEL was 20 mg/kg/day based on the observed inhibition of brain and RBC cholinesterase activities at 50 mg/kg/day. The NOEL for local dermal effects was 50 mg/kg/day, corresponding to an approximate dermal dose of 0.31 mg/cm^2 (assuming a body surface area for a 200 g rat of 325 cm^2 - Harkness and Wagner [1983]), based on the atonia noted at 100 mg/kg/day.

This study was considered to be supplemental.

Table III-5. RBC and brain cholinesterase activities in a 4-wk carbaryl repeat-dose dermal study in Sprague-Dawley rats (Austin, 2002a)

	Carbaryl dose, males (mg/kg)				Carbaryl dose, females (mg/kg)			
	0	20	50	100	0	20	50	100
RBC, Umol/L - Pre-daily dose assays								
Day -4	1283±82.3	1272±71.1 99%	1338±96.5 104%	1305±91.8 102%	1323±91.9	1302±96.9 98%	1300±131.4 98%	1299±90.5 98%
Day 1	1334±95.1	1373±124.9 103%	1382±121.9 104%	1326±102.2 99%	1410±80.6	1398±74.2 99%	1513±143.2 107%	1405±123.6 100%
Day 8	1136±82.2	1081±135.3 95%	1150±123.4 101%	1012±51.3* 89%	1075±81.5	1144±94.1 106%	1125±186.9 105%	1199±135.4 112%
Day 15	1162±116.7	1183±133.5 102%	1221±104.3 105%	1194±103.3 103%	1172±117.2	1146±87.1 98%	1252±77.7 107%	1254±70.2 107%
Day 22	1273±90.1	1304±150.9 102%	1269±89.7 100%	1113±85.6* 87%	1362±92.4	1291±120.1 95%	1215±142.5 89%	1232±142.4 90%
RBC, Umol/L - Post-daily dose assays								
Day 5	1281±99.0	1308±113.8 102%	1122±63.2* 88%	1089±79.4* 85%	1339±120.5	1363±108.0 102%	1165±116.4* 87%	1172±177.4* 88%
Day 12	941±111.4	918±114.1 98%	851±83.7 90%	740±92.9* 79%	996±91.4	961±68.5 96%	801±111.7* 80%	865±120.2* 87%
Day 19	1199±142.3	1191±110.7 99%	1164±112.7 97%	1002±118.8* 84%	1211±101.2	1330±97.3 110%	1199±115.6 99%	1188±282.3 98%
Day 26	1266±123.7	1360±123.8 107%	1280±146.1 101%	1282±170.4 101%	1465±133.2	1412±144.4 96%	1394±146.4 95%	1492±219.7 102%
Brain, Umol/mg - Post-daily dose assays								
Day 26	40±4.8	41±3.8 103%	34±4.0* 85%	34±7.1* 85%	45±2.9	45±4.4 100%	41±4.4 91%	34±6.0* 76%

*p<0.05

In a continuation of the repeat-dose dermal studies done for this series, Austin (2002b) applied Sevin XLR Plus (44.82% carbaryl) to ~10% of the body surface of Sprague-Dawley rats, 8/sex/group, at 0, 20, 50 or 100 µl/kg/day, 6-7 hr/day, 5 days/wk, for 4 weeks. Body weight, body weight change, food consumption and dermal irritation were evaluated and were negative for treatment-related effects. RBC cholinesterase was measured before daily exposure on days 1, 8, 15 and 22, and within 1 hr after dose removal on days 5, 12, 19 and 26. Brain cholinesterase activity was not measured.

High dose females showed a 12% inhibition of RBC cholinesterase activity compared to controls ($p \leq 0.05$) on days 5 and 12 after dosing, but not on days 19 and 26. Clear test article-induced inhibition was not detected in males.

The systemic and dermal irritation NOELs were >100 µl/mg/day. The RBC cholinesterase NOEL was not determined in light of the mildness and inconsistency of the

cholinesterase data.

This study was considered supplemental.

Austin (2002c) applied Sevin 80S (80.07% carbaryl) to ~10% of the body surface of Sprague-Dawley rats, 8/sex/group, at doses of 0, 20, 50 or 100 mg/kg/day, 6-7 hr/day, 5 days/wk, for 4 weeks. The material was applied as a powder to moistened skin and covered. Body weight, food consumption, dermal irritation and clinical signs were monitored. There were no treatment-related findings. RBC cholinesterase activity was measured pretest, before dosing on days 1, 8, 15 and 22, and within 1 hr after removal of the dosing material on days 5, 12, 19 and 26. Brain cholinesterase was not measured. Necropsies were not performed.

RBC cholinesterase activity was inhibited by 8-20% at 50 and 100 mg/kg when samples were taken within the hour after dosing ($p \leq 0.05$). No consistent pattern of inhibition was noted with samples taken before the daily dose.

The NOEL for systemic effects in this study was >100 mg/kg/day. The NOEL for RBC cholinesterase inhibition was 20 mg/kg/day based on the effects noted at 50 mg/kg/day.

This study was considered to be supplemental.

D. CHRONIC TOXICITY AND ONCOGENICITY

1. Overview

Studies of the toxicologic consequences of chronic carbaryl exposure in rats and mice provided evidence for oncogenicity in several tissues. Most importantly from a risk assessment perspective, carbaryl induced vascular tumors called hemangiosarcomas (and hemangiomas) in a dose-dependent fashion in male mice, enabling the calculation of an oncogenic potency value for carbaryl. Less clear, but also possibly dose-dependent, was the induction of hepatocellular adenomas and carcinomas in female mice. Tumor development was also evident in male mouse kidneys and rat urinary bladder, liver and thyroid. Non-oncogenic effects, including cholinesterase inhibition and a diverse array of adverse signs were recorded in various tissues. One clearly adverse sign, cataracts, was noted in both rats and mice at the high dose in each of those respective studies. While cholinesterase inhibition occurred in the 1-year dog study, no unusual clinical signs were noted.

Chronic NOELs and LOELs are summarized in Table III-9.

2. Laboratory animal studies

Rats. Hamada (1993a) exposed Sprague-Dawley rats to dietary carbaryl (purity, 99%) for 2 years. The doses were 0, 250, 1500 and 7500 ppm, corresponding to mean systemic doses of 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. There were 90 rats/sex in the control and high dose groups, and 80 rats/sex in the low and mid dose groups. Examined parameters included mortality, clinical signs, body weights, food consumption, hematology, blood chemistry, ophthalmology, gross pathology, histopathology and organ weights. Interim sacrifices on 10/sex/dose were carried out at 26 and 52 weeks. Another 10/sex from the control and high dose groups were reestablished on basal diet for 4 weeks between weeks 53 and 57. Finally, 10 rats/sex/group were subjected to clinical laboratory exams after 78 and 104 weeks and sacrificed at study termination with the remaining animals.

There was no effect of carbaryl on mortality. Survival rates at termination were 60%, 45%*, 44%* and 61% for males and 33%, 40%, 40% and 69%* for females (*$p<0.05$). The biological significance of the increased female survival at the high dose was unclear.

The following clinical signs were likely induced by carbaryl, particularly at the high dose (but not excluding the possibility that there were lower-dose effects): hunched posture (♂: 2/90, 3/80, 3/80, 8/90), limited use of hind limbs (♂: 0/90, 2/80, 0/80, 8/90), alopecia-front limbs (♀: 5/90, 8/80, 8/80, 21/90), alopecia-front feet (♀: 2/90, 4/80, 9/80, 20/90), alopecia-multiple sites (♀: 0/90, 0/80, 2/80, 5/90), urine stains (♂: 3/90, 7/80, 2/80, 21/90; ♀: 3/90, 10/80, 12/80, 23/90).

Statistically significant decrements in body weight were noted at the mid and high doses in both sexes. Such effects in mid dose males were less evident after week 18. Statistically significant weight decrements were also noted in low dose males at weeks 4, 17 and 21, though the infrequency of this observation cast question on its toxicologic significance. By week 105, the high dose males and females weighed 65% and 55% of controls, respectively, both statistically significant at the 0.05 level. Mid dose animals showed decrements of 9% in males (not significant) and 18% in females ($p<0.05$) at week 105.

While weekly food consumption differentials never achieved statistical significance, *total* consumption over the 2-year period was reduced by a statistically significant margin at the high dose. As the high dose body weight decrements clearly exceeded the corresponding decrements in food consumption, the body weight effects were considered evidence of systemic toxicity. Recovery animals (dosing ceased after 52 weeks) showed greater body weights than their main-study counterparts by week 57, though they were still less than the unexposed

controls. The body weight and food consumption effects for the main study animals are summarized in Table III-6a.

Ophthalmoscopic exams at week 104 revealed a rise in cataracts at the high dose in both genders, though the total number of animals examined was not stated (unilateral + bilateral cataract incidence, ♂: 4, 6, 7, 12; ♀: 3, 2, 4, 10). The study ophthalmologist considered these to be incidental. However, because of the consistent rise at the high dose in both sexes and the clear effect seen in mice (Hamada, 1993b; see below), cataracts were considered to be caused by carbaryl for the purposes of this assessment.

Several hematologic parameters were altered by statistically significant margins at the high dose, particularly in males. These included hemoglobin (⇑ wk. 57), hematocrit (⇑ wk. 57), mean cell volume (⇑ wk. 27), mean cell hemoglobin (⇑ wks. 27 & 53), mean cell hemoglobin concentration (⇑ wks. 27 & 57 in ♂; ⇓ wk. 57 in ♀), leukocyte count (⇓ wk. 27, including mid dose), corrected leukocyte count (⇓ wks. 27 & 79), lymphocytes (⇓ wks. 27, 53, 79) and eosinophils (⇑ wk. 27). These changes were not considered to carry great toxicologic significance, and may be secondary to other changes.

Clinical chemistry also revealed statistically significant changes at the high dose in both sexes. These included blood urea nitrogen (♀, ⇑ wk. 79), creatinine (♂, ⇓ wk. 53, including mid dose), total cholesterol (♂ & ♀, ⇑ wk. 27; ♂, ⇑ wks. 53, 79, 105), aspartate aminotransferase (♂ & ♀, ⇓ wk. 27; ♂, ⇓ wk. 53), alanine aminotransferase (♀, ⇓ wks. 27, 53), total protein (♂, ⇑ wk. 57), creatine kinase (♂, ⇓ wk. 53) and sodium (♂, ⇑ wk. 53). The toxicologic significance of these changes was unclear.

Cholinesterase measurements revealed statistically significant decrements at the following times: plasma ChE in high dose males & females, wks. 26 & 52 and in high dose females, wks. 78 & 104; RBC ChE in mid and high dose females, wks. 52, 78 & 104 and in high dose males, wks. 52, 78 & 104; brain ChE, mid and high dose females, wks. 53 & 105, mid and high dose males, wk. 53 and high dose males, wk. 105. The highest level of statistically significant inhibition of plasma ChE was 57% (♀, wk. 78). The highest level of statistically significant inhibition of RBC ChE was 38% (♀, wk. 104). The highest level of statistically significant inhibition of brain ChE was 31% (♀, wk. 53). Inhibition of brain ChE at the mid dose reached 16% in females at week 105 and 10% in males at week 53. ChE activities appear in Table III-6b.

Urinalysis data were not provided. However, the report states that they were “generally comparable between control and treated groups”, but with increased incidences of dark urine at the mid and high doses, and occult blood and increased erythrocytes at the high dose. These changes were described as “mild, were not accompanied by evidence of renal compromise in the biochemical data, and cannot be definitively attributed to the administration of the test material.” (p. 139)

Most of the statistically significant organ weight changes were recorded as changes relative to brain or terminal body weights at the high dose. Statistically significant mid dose changes were all relative. Statistically significant absolute changes occurred in week 53 female kidney (2.56, 2.47, 2.64 and 2.22* g) and liver (12.14, 12.21, 13.71, 10.23* g), and week 105 female kidney (3.34, 3.14, 3.27, 2.80* g).

Gross pathologic changes were largely restricted to high dose animals at terminal sacrifice. These included pale areas in the lung (♂: 0/40, 0/31, 0/31, 4/43; ♀: 0/22, 0/27, 0/28, 4/46), pale areas in the liver (♂: 0/40, 0/31, 1/31, 4/43; ♀: 1/22, 0/27, 0/28, 0/46), and masses in the urinary bladder (♂: 0/40, 0/31, 0/31, 2/43; ♀: 0/22, 0/27, 0/28, 4/46; masses discovered during histologic processing, ♂: 0/40, 0/31, 0/31, 6/43; ♀: 0/22, 0/27, 0/28, 4/46).

By the terminal sacrifice, both neoplastic and non-neoplastic histopathologic changes were evident in several organs. These are summarized in Table III-6c and discussed in the

following paragraphs.

Urinary bladder: There was a pronounced increase in hyperplasia at the high dose, with hints of an increase at the mid dose, particularly in females at terminal sacrifice. Also occurring at the high dose were increased incidences of benign transitional cell papillomas and malignant transitional cell carcinomas, squamous metaplasia, high mitotic index and atypia. The neoplastic and hyperplastic observations were often coincident, suggesting that the hyperplasia was preneoplastic. The report described the transitional cell papillomas and carcinomas as exophytic [6]. According to the report (p. 53), the carcinomas "exhibited many of the following microscopic features: (1) nuclear and cytologic atypia, (2) hyperchromasia, (3) orientation into dense sheets, with loss of normal differentiation, (4) high mitotic index, (5) squamous metaplasia, and (6) stalk invasion. No evidence of metastasis was present."

Kidney: While hyperplasia of the renal transitional epithelium was a common occurrence in all of the animals, this character was also increased in high dose males. One high dose male exhibited a transitional cell carcinoma which was judged to be due to treatment.

Liver: The incidence of hepatocellular adenomas was increased in high dose females. Hepatocytic hypertrophy, which increased at the high dose in both sexes, was described as "generally centri- to mid-lobular and was graded minimal to slight in most animals" (p. 54). High dose females exhibited an increased incidence of pigment, which was primarily localized to hepatocytes and, to a lesser extent, to reticuloendothelial cells. The report describes this pigment as "morphologically compatible with lipofuscin [7]" (p. 54). Eosinophilic foci were also increased in high dose females, as were intracytoplasmic hyaline inclusions, described as having "a vacuolated center surrounded by an outer eosinophilic lamellar coat" (p. 55), in high dose males.

Thyroid: The incidence of follicular cell hypertrophy increased greatly in high dose females and slightly in high dose males. The report describes this change as "graded minimal to slight and was morphologically characterized by an increased height of follicular epithelium with an associated decreased colloid" (p. 55). An increase in benign follicular cell adenomas was noted in high dose males, in addition to a single high dose male exhibiting a follicular cell carcinoma. The adenomas were described as "comprised of well-differentiated cells exhibiting a follicular growth pattern. Cystic dilatation was often present within the adenomas" (p. 55).

Lung: The incidence of focal pneumonitis and alveolar foamy macrophages, changes that were correlated with the observation of pale foci at necropsy (see above), rose at the high dose in both sexes. As stated in the report, "alveolar foamy macrophages were most severe in the Group 4 [high dose] females and was characterized by multifocal distribution of relatively large pale to eosinophilic alveolar macrophages throughout the pulmonary parenchyma. In many animals, this macrophage infiltrate was relatively dense and was associated with a mixed inflammatory cell interstitial infiltrate, resulting in a focal pneumonitis" (p. 56). A low incidence of alveolar hyperplasia was also noted in high dose females.

Pancreas: High dose females showed an increased incidence of acinar cell vacuolization, described by the report as "multifocal in distribution, graded minimal in most animals, and was characterized by the presence of numerous oval cytoplasmic vacuoles that

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[6]Exophytic growth is defined in oncology as "proliferating on the exterior or surface epithelium of an organ or other structure, in which the growth originated" (Dorland's Illustrated Medical Dictionary, 26th Edition, 1985; W.B. Saunders Company; p. 475)

[7] Lipofuscin: "any one of a class of fatty pigments formed by the solution of a pigment in fat" (Dorland's Illustrated Medical Dictionary, 26th Edition, 1985; W.B. Saunders Company; p. 750)

are morphologically compatible with fat" (p. 57). A low incidence of benign acinar cell adenomas in high dose females was also observed.

Sciatic nerve and adjacent muscle: An increase in sciatic nerve degeneration was evident at the high dose despite the observation that the lesion was common even among control animals (an indication that it evolves as a natural function of aging). According to the report, "sciatic nerve lesions in all groups were morphologically compatible with the peripheral nerve neuropathy commonly seen in aged Sprague-Dawley rats. However, there was more extensive microscopic evidence of myelin degeneration, macrophage infiltration, eosinophilic globular formations, axonal loss, and fibrosis in the Group 4 [high dose] males and female rats, resulting in higher severity grades for degeneration" (p. 57). Degeneration in the adjacent skeletal muscle, likely a function of the effect on nerve, also rose at the high dose among unscheduled death animals.

Seminal vesicle: The biological significance of the decreased seminal vesicle secretion was not clear and may have been incidental.

The NOEL for non-oncogenic systemic effects was established at 250 ppm (10.0-12.6 mg/kg/day) based on (1) the inhibition of brain cholinesterase at 1500 ppm (60.2-78.6 mg/kg/day) and (2) the 18% overall inhibition in female weight gain at 1500 ppm over the 2-year period. Based on the evidence for neoplasia in the urinary bladder, liver and thyroid, carbaryl is clearly carcinogenic in rats. Tumor induction was seen mainly at the high dose, which - based on the large body weight decrements, clinical signs and statistically significant plasma, RBC and brain cholinesterase inhibition - exceeded the MTD. Hepatocellular adenomas may also have increased in mid dose females, though in a non-statistically significant manner. The mid dose approached an MTD in this study based on body weight decrements (9% and 18% in males and females, respectively, at week 105) and statistically significant RBC and brain cholinesterase inhibition.

This study was considered to be acceptable by FIFRA standards.

Table III-6a. Body weights and food consumption in Sprague-Dawley rats exposed over a 2-yr period to dietary carbaryl (Hamada, 1993a)

	Carbaryl dose (ppm), males [a]				Carbaryl dose (ppm), females [a]			
Time	**0**	**250**	**1500**	**7500**	**0**	**250**	**1500**	**7500**
Body weight (g)								
Week 1	235	234	236	235	179	176	178	175*
Week 4	385	376*	368*	299*	244	242	236*	201*
Week 17	586	569*	569*	442*	331	327	315*	243*
Week 21	620	601*	595*	457*	344	342	326*	245*
Week 53	724	716	708	526*	431	425	410*	264*
Week 79	745	736	718	523	470	463	437	281
Week 105	717	692	677	463*	510	501	450*	278*
Food consumption (g) weeks 1-102	6568	6385	6373	5407*	4918	4938	4955	4144*

* $p<0.05$

[a] Mean systemic doses: 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

Table III-6b. Cholinesterase activities for Sprague-Dawley rats exposed to dietary carbaryl for 2 years (Hamada, 1993a)

	Carbaryl dose, males (ppm) [a]				Carbaryl dose, females (ppm) [a]			
Time	**0**	**250**	**1500**	**7500**	**0**	**250**	**1500**	**7500**
Week -1								
plasma	2.8 [b]	n/a [c]	n/a	n/a	4.8	n/a	n/a	n/a
RBC	5.6				5.6			
brain	68.1				69.6			
Week 26								
plasma	2.2	2.1 (5) [d]	2.1 (5)	1.6* (27)	12.9	12.6 (2)	10.7 (17)	6.1* (53)
RBC	5.9	5.6 (5)	5.3 (10)	4.8 (19)	5.7	5.1 (11)	5.0 (12)	4.3* (25)
brain	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Week 52								
plasma	3.0	2.8 (7)	2.6 (13)	1.8* (40)	11.2	11.2 (0)	9.2 (18)	4.9* (56)
RBC	6.0	5.7 (5)	4.8* (20)	4.6* (23)	5.8	5.1 (12)	4.3* (26)	3.7* (36)
brain (wk 53)	51.3	50.7 (1)	46.1* (10)	37.1* (28)	51.5	51.8 (+1)	44.8* (13)	35.6* (31)
Week 78								
plasma	3.4	3.1 (9)	4.0 (+29)	2.1 (38)	10.1	9.5 (6)	8.2 (19)	4.3* (57)
RBC	6.2	5.5 (11)	4.8* (23)	3.9* (37)	5.6	5.3 (5)	4.4* (21)	3.9* (30)
brain	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Week 104								
plasma	4.0	6.4 (+60)	3.7 (7)	2.3 (42)	7.6	7.0 (8)	7.2 (5)	4.1* (46)
RBC	5.7	6.6 (+16)	6.0 (5)	4.0* (30)	5.8	5.4 (7)	4.5* (22)	3.6* (38)
brain (wk 105)	54.4	55.1 (+1)	53.1 (2)	49.5* (9)	57.4	53.5 (7)	48.4* (16)	44.8* (22)

* $p<0.05$

[a] Mean systemic doses: 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.

[b] Plasma and RBC ChE activities expressed as µmol/ml; brain ChE activity expressed as µmol/g.

[c] n/a, not applicable

[d] Numbers in parentheses represent the percentage of inhibition compared to concurrent controls.

Table III-6c. Neoplastic and non-neoplastic changes in Sprague-Dawley rats exposed over a 2-yr period to dietary carbaryl (Hamada, 1993a)

		Carbaryl dose, males (ppm) [a]				Carbaryl dose, females (ppm) [a]			
		0	**250**	**1500**	**7500**	**0**	**250**	**1500**	**7500**
Urinary bladder									
hyperplasia	T [b]	3/40^{+++}	1/31	4/31	39/43***	0/22^{+++}	0/26	3/28	42/45***
	U [b]	5/30^{+++}	7/39	6/39	15/28**	6/47^{+++}	6/43	3/41	14/24***
transitional cell papilloma (B [c])	T	0/40^{+++}	0/31	0/31	10/43***	0/22^{++}	0/26	0/28	5/45
	U	0/30^{++}	0/39	0/39	2/28	1/47^{+++}	0/43	0/41	2/24
	C [g]	0/68^{+++}	0/67	0/70	12/69***	1/67^{+++}	0/69	0/68	7/67*
transitional cell carcinoma (M [c])	T	0/40^{+++}	0/31	0/31	9/43**	0/22^{++}	0/26	0/28	5/45
	U	0/30^{++}	0/39	0/39	2/28	0/47^{+}	0/43	0/41	1/24
	C [h]	0/68^{+++}	0/67	0/70	11/69***	0/67^{+++}	0/69	0/68	6/67*
trans. papilloma + carcinoma [f]		**0/67^{+++}**	**0/67**	**0/70**	**22/69*** **	**1/67^{+++}**	**0/69**	**0/68**	**13/67*** **
squamous metaplasia	T	0/40^{+++}	0/31	0/31	6/43*	0/22^{+}	0/26	0/28	3/45
	U	0/30^{+}	0/39	0/39	1/28	0/47	0/43	0/41	0/24
high mitotic index	T	0/40^{+++}	0/31	0/31	10/43***	0/22^{++}	0/26	0/28	4/45
	U	0/30^{++}	0/39	0/39	2/28	0/47	0/43	0/41	0/24
atypia	T	0/40^{+++}	0/31	0/31	5/43*	0/22^{+++}	0/26	0/28	13/45**
	U	0/30^{+++}	0/39	0/39	3/28	1/47	0/43	0/41	1/24
invasion	T	0/40^{+}	0/31	0/31	2/43	0/22	0/26	0/28	0/45
	U	0/30	0/39	0/39	0/28	0/47	0/43	0/41	0/24
Kidney									
hyperplasia, transitional epith.	T	8/40^{+++}	4/31	6/31	22/43**	8/22	18/27	6/28	15/46
	U	4/30^{++}	3/38	3/39	8/28	13/48	23/43	22/42	6/24
transitional cell carcinoma (M)	T	0/40	0/31	0/31	1/43	0/22	0/27	0/28	0/46
	U	0/30	0/38	0/39	0/28	0/48	0/43	0/42	0/24
suppurative pyelonephritis	T	0/40	1/31	0/31	0/43	0/22^{+}	0/27	0/28	2/46
	U	1/30	0/38	1/39	1/28	2/48	0/43	1/42	0/24
pelvis pigment	T	0/40^{+}	0/31	0/31	2/43	0/22	0/27	0/28	0/46
	U	0/30^{+}	0/38	0/39	1/28	0/48	0/43	0/42	0/24

Liver									
pigment	T	0/40	0/31	1/31	1/43	$0/22^{+++}$	0/27	1/28	16/46***
	U	2/31	3/39	4/39	3/28	$6/48^{++}$	6/43	6/42	9/24*
hepatocellular adenoma (B)	T	0/40	1/31	2/31	1/43	$0/22^{+}$	0/27	2/28	4/46
	U	1/31	0/39	0/39	0/28	$1/48^{++}$	0/43	1/42	3/24
	C [i]	**1/66**	**1/67**	**3/69**	**1/67**	**$1/64^{+++}$**	**0/70**	**3/69**	**7/68***
hepatocyte hypertrophy	T	$0/40^{+++}$	1/31	1/31	30/43***	$2/22^{+++}$	4/27	2/28	23/46***
	U	4/31	2/39	3/39	5/28	$7/48^{+++}$	3/43	7/42	14/24**
ic[4] hyaline inclusions	T	$0/40^{+++}$	0/31	0/31	$12/43^{+++}$	0/22	0/27	0/28	0/46
	U	$0/31^{+}$	0/39	0/39	1/28	0/48	0/43	0/42	0/24
eosinophilic cellular alteration	T	5/40	5/31	4/31	7/43	$4/22^{+}$	5/27	4/28	15/46
	U	3/31	0/39	2/39	0/28	$2/48^{+}$	2/43	4/42	4/24
Thyroid									
hypertrophy	T	1/40	0/31	0/31	2/43	$3/22^{+++}$	3/27	2/28	30/46***
	U	$0/31^{+++}$	1/39	2/39	6/28**	$1/48^{++}$	1/43	0/42	3/24
follicular cell adenoma (B)	T	$0/40^{++}$	2/31	0/31	6/43*	0/22	0/27	0/28	1/46
	U	$0/31^{++}$	0/39	0/39	2/28	0/48	0/43	0/42	0/24
	C [j]	$0/66^{+++}$	2/67	0/69	8/67**	064^{+}	0/70	0/69	1/68
follicular cell carcinoma (M)	T	0/40	0/31	0/31	1/43	1/22	0/27	0/28	0/46
	U	0/31	0/39	0/39	0/28	0/48	0/43	0/42	0/24
	C [k]	$0/66^{+}$	0/67	0/69	1/67	0/64	0/64	0/69	0/68
follic. adenoma + carcinoma [l]		**$0/66^{+++}$**	**2/67**	**0/69**	**9/67****	**$0/64^{+}$**	**0/70**	**0/69**	**1/68**
Lung									
focal pneumonitis	T	6/40	3/31	4/31	10/43	$3/22^{+++}$	4/27	3/28	35/46***
	U	$1/31^{++}$	0/39	2/39	4/28	$5/48^{+++}$	2/43	0/42	7/24*
alveolar foamy macrophages	T	$11/40^{+++}$	8/31	9/31	26/43**	$4/22^{+++}$	3/27	5/28	42/46***
	U	$2/31^{+++}$	3/39	10/39*	12/28**	$9/48^{+++}$	3/43	9/42	16/24***
alveolus hyperplasia	T	1/40	0/31	0/31	0/43	$0/22^{+}$	0/27	0/28	2/46
	U	0/31	0/39	0/39	0/28	0/48	0/43	0/42	0/24

Pancreas									
acinar cell adenoma (B)	T	0/40	0/0	0/0	0/43	0/22^{+}	0/25	0/27	2/46
	U	0/30	0/37	0/39	0/28	0/48	0/43	0/42	0/24
acinar cell vacuolization	T	0/40	0/0	0/0	0/43	0/22^{+++}	0/25	0/27	15/46**
	U	0/30	1/37	0/39	0/28	0/48^{+++}	0/43	2/42	5/24**
Sciatic nerve & adjacent muscle									
nerve degeneration [e]	T	34/40^{+}	31/31*	31/31*	42/42*	22/22	24/25	26/27	44/45
	U	10/31^{++}	11/36	21/39	17/27*	19/48^{++}	15/42	18/42	17/23**
muscle degeneration	T	2/40	2/31	6/31	5/43	0/22	0/26	1/28	2/45
	U	2/31^{+++}	2/39	4/39	8/28*	0/48^{+++}	0/43	2/42	4/24*
Seminal vesicle									
decreased secretion	T	1/40	1/2	1/2	2/43	n/a [d]	n/a	n/a	n/a
	U	2/31^{++}	3/39	5/39	8/28*				

*,**,***: p<0.05, 0.01, 0.001 (Fisher Exact Test) - tests performed by risk assessor.
$^{+}$,$^{++}$,$^{+++}$: p<0.05, 0.01, 0.001 (Cochran-Armitage Trend Test) - tests performed by risk assessor.

[a] Mean systemic doses: 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.
[b] T, terminal sacrifice; U, unscheduled deaths
[c] B, benign; M, malignant
[d] ic, intracytoplasmic; n/a, not applicable
[e] While incidence rates for sciatic nerve degeneration were similar among dose groups, the severity of this parameter increased at the high dose in both sexes (see text).
[f] Sum of "at risk" urinary bladder transitional cell papillomas and transitional cell carcinomas.

[g] Combined "at risk" urinary bladder transitional cell papilloma incidence rate from terminal sacrifices and unscheduled deaths. The number of animals at risk was determined by excluding those animals that died before week 42 (which is the point when the first unscheduled death occurred with a transitional cell papilloma). The number of males dying before week 42 was 3, 3, 0 and 2 at ascending doses, making the number of "at risk" males equal to 67, 67, 70 and 69. The number of females dying before week 42 was 2, 0, 1 and 2, making the number of "at risk" females equal to 67, 69, 68 and 67.

[h] Combined "at risk" urinary bladder transitional cell carcinoma incidence rate from terminal sacrifices and unscheduled deaths. The number of animals at risk was determined by excluding those animals that died before week 42 (which is the point when the first unscheduled death occurred with a transitional cell papilloma, considered to be a benign precursor for the transitional cell carcinoma). The number of males dying before week 42 was 2, 3, 0 and 2 at ascending doses, making the number of "at risk" males 68, 67, 70 and 69. The number of females dying before week 42 was 2, 0, 1 and 2, making the number of "at risk" females 67, 69, 68 and 67.

[i] Combined "at risk" hepatocellular adenoma incidence rate from terminal sacrifices and unscheduled deaths. The number of animals at risk was determined by excluding those animals that died before week 53 - the first unscheduled death of an animal with hepatocellular adenoma occurred in week 78. It was thus considered that any unscheduled deaths before 1 year were not likely to show hepatocellular adenoma. The number of males dying before week 53 was 5, 3, 1 and 4 at ascending doses, making the number of "at risk" males 66, 67, 69 and 67. The number of females dying before week 53 was 6, 0, 1 and 2, making the number of "at risk" females 64, 70, 69 and 68.

[j] Combined "at risk" thyroid follicular adenoma incidence rate from terminal sacrifices and unscheduled deaths. The number of animals at risk was determined by excluding those animals that died before week 53 - the first unscheduled death of an animal with a thyroid follicular adenoma occurred in week 93. It was thus considered that any unscheduled deaths before 1 year were not likely to show thyroid follicular adenomas. The number of males dying before week 53 was 5, 3, 1 and 4 at ascending doses, making the number of "at risk" males 66, 67, 69 and 67. The number of females dying before week 53 was 6, 0, 1 and 2, making the number of "at risk" females 64, 70, 69 and 68.

[k] Combined "at risk" thyroid follicular carcinoma incidence rate from terminal sacrifices and unscheduled deaths. The number of animals at risk was determined by excluding those animals that died before week 53 - the first unscheduled death of an animal with a thyroid follicular adenoma (considered a benign precursor to thyroid follicular carcinoma) occurred in week 93. It was thus considered that any unscheduled deaths before 1 year were not likely to show thyroid follicular adenomas. The number of males dying before week 53 was 5, 3, 1 and 4 at ascending doses, making the number of "at risk" males 66, 67, 69 and 67. The number of females dying before week 53 was 6, 0, 1 and 2, making the number of "at risk" females 64, 70, 69 and 68.

[l] Sum of "at risk" thyroid follicular adenomas and thyroid follicular carcinomas.

Mice. Hamada (1993b) exposed 80 CD-1 mice/sex/dose to dietary carbaryl (purity, 99.3%) for two years. The doses were 0, 100, 1000 and 8000 ppm, corresponding to average systemic doses of 0, 14.73, 145.99 and 1248.93 mg/kg/day in males and 0, 18.11, 180.86 and 1440.62 mg/kg/day in females. Animals were examined for mortality, clinical signs, body weights, food consumption, hematology, blood chemistry, ophthalmology, gross pathology, histopathology and organ weights. Ten mice/sex/dose were sacrificed at one year for interim gross and histopathologic exams and for organ weight determinations.

Six high dose females died during the first 7 weeks of dosing, compared to one control female and no females at either the low or mid doses, respectively. There were no further differences in mortality between controls and treated animals, including males. After week 70, animals in all groups began to die more quickly, though without an overt influence of dose. By the end of week 104, 54, 52, 53 and 44% of the males and 50, 50, 46 and 49% of the females survived. The early high dose female deaths were accompanied during the first 3 weeks by the appearance of thinness and hunched posture in ~40% of the females in that group (and in some of the males). Interestingly, this parameter was noted only sporadically after 6 weeks, though there was an increased incidence, particularly at the high dose, during the final 6 months of the study. Other clinical observations that occurred with higher frequency at the high dose included languidity, urine staining, pale body, opaque eyes, rough haircoat, dyspnea, polypnea, and few or no feces. The latter four signs also were increased at the mid dose, though only in males for the latter three signs.

High dose animals exhibited a prominent body weight decrement, apparent from the first study week through the end of the study. Weight losses were apparent during the first two weeks in both sexes. By the end of 4 weeks, high dose males had gained only 19% of controls, while high dose females had gained only 30% of controls. Mean body weights at week 52 were 38.7, 38.5, 37.6 and 34.2* g in males (high dose males had gained 66% of controls) and 32.9, 32.8, 32.7 and 29.5* g in females (high dose females had gained 77% of controls) (*$p<0.05$). By termination at week 104, mean body weights were 36.7, 37.4, 37.9 and 32.4 g in males (high dose males had gained 62% of controls) and 32.3, 33.1, 32.5, and 28.0 g in females (high dose females had gained 68% of controls). Small statistically significant weight differences were also noted at the low and mid doses, particularly in males during the first 4 weeks. However, these were not convincingly due to carbaryl exposure since the rate of weight gain was virtually equivalent to the controls. Weekly food consumption was consistently statistically reduced in high dose females (analyses were performed at weeks 13, 26, 50, 78 and 102 only). Consumption in mid and high dose males was notably reduced after week 73 (statistically significant at week 78).

Reductions were observed in RBC counts, hemoglobin and hematocrit in high-dose animals, with statistical significance recorded at week 53 in females and week 105 in males (these were the only two measurement times). Platelet counts were statistically increased in high dose females at weeks 53 (1408, 1303, 1383 and 1779* th/µl) and 105 (845, 957, 1185, 1568* th/µl), as were lymphocyte, corrected white blood cell and eosinophil counts at week 53. Because of their consistency (RBC parameters) and magnitude (platelet counts in females), these changes were considered due to carbaryl exposure, though a mechanism is not known.

Statistically significant reductions in RBC cholinesterase activities were recorded in mid and high dose males at week 53 (7.3, 7.0, 5.6*, 5.1* µmol/ml). Statistically significant reductions in brain cholinesterase activities were noted for mid and high dose males and females at week 53 (♂: 86.0, 81.3, 70.7*, 36.9*; ♀: 84.1, 81.1, 73.5*, 44.6* µmol/g) and for mid and high dose males and high dose females at week 105 (♂: 59.9, 59.4, 52.0*, 35.9* µmol/g; ♀: 62.2, 58.7, 55.2, 41.0* µmol/g). The depressed activities at the low dose were possibly carbaryl related, though the small decrement and lack of statistical significance made this unclear. In addition,

clinical signs were not apparent at the low dose.

With the exception of an increase in internal eye opacity in high dose female unscheduled deaths (1/33, 1/39, 0/33, 4/40), neither unscheduled deaths nor interim sacrifices revealed carbaryl-related pathologies. Necropsies performed on terminal sacrifice animals revealed the following effects, mostly at the high dose: kidney mass (♂: 0/37, 0/31, 0/37, 3/30), enlarged seminal vesicle (♂: 15/37, 12/31, 12/37, 1/30), uterine mass (♀: 8/34, 4/31, 5/32, 0/32), uterine cyst (♀: 17/34, 14/31, 20/32, 6/32), and internal eye opacity (♂: 1/37, 2/31, 1/37, 4/30; ♀: 2/34, 5/31, 2/32, 16/32).

Weight differentials were noted at wk 105 in several organ systems at the high dose. These included lung (statistically decreased absolute weight at the high dose and relative weights at the mid and high doses in females), liver / gall bladder (statistically increased relative weights at the high dose, both genders) and kidney (statistically increased relative weights at the high dose, both genders). Similar changes were evident at the wk 53 interim sacrifice. Absolute and relative high dose ovary weights were statistically suppressed at interim sacrifice, though not at terminal sacrifice. These organ weight changes are summarized in Table III-7a.

Several non-neoplastic and neoplastic histopathologic changes were evident, with some noted as early as the 1-year interim sacrifice. These are recounted by tissue in the following paragraphs and in Tables III-7b and III-7c.

Urinary bladder: The superficial transitional epithelium (umbrella cells) exhibited an increased incidence of eosinophilic intracytoplasmic protein-like droplets at the mid and high doses. These were evident as early as the interim (52-wk) sacrifices. No accompanying degeneration, necrosis, inflammation or proliferation was noted. The toxicologic significance of this sign was not known.

Eye: There was an increased incidence of animals bearing bilateral cataracts at the high dose, though this character occurred at a relatively high frequency even among controls. The incidence of unilateral cataracts was not clearly affected.

Spleen: The incidence of splenic pigmentation rose precipitously at the high dose among interim sacrifices of both genders. By the time of the terminal sacrifices (wk. 104) there were no differences in incidence of this character. However, a “slight” increase in severity at the mid and high doses was noted (severity data not presented in Table III-7b). Increased extramedullary hematopoiesis was noted among high dose interim sacrifices, and exhibited slightly increased severity at terminal sacrifice (though incidence was similar to controls). The report suggests that both of these parameters reflected an increased splenic turnover of red blood cells with secondary extramedullary hematopoiesis. The RBC-related hematologic changes noted above may also be related to these splenic effects.

Duodenum, colon, testis: The incidence of amyloidosis[8] increased at the high dose in these organs among the unscheduled deaths. No association with dose was found with the terminal sacrifices, however. The toxicologic significance of this observation was not clear, though it is noted that amyloidosis was listed as a prominent cause of death in this study.

Gallbladder: The incidence of subacute inflammation of the gallbladder increased among terminal sacrifices at all doses. The toxicologic significance of this observation was unclear.

Oncogenic observations are summarized in the following paragraphs. Carbaryl had clear

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[8]Amyloidosis: “the accumulation of amyloid [‘an abnormal complex material, most probably a glycoprotein...’] in various body tissues, which, when advanced, engulfs and obliterates parenchynmal cells and thus injures the affected organ.” (Dorland’s Illustrated Medical Dictionary, 26th Edition, 1985; W.B. Saunders Company; p. 64)

oncogenic effects, as noted below in vascular tissue (many organs), kidney and liver.

Vascular tissue: An increased incidence of vascular neoplasms, identified as hemangiomas and hemangiosarcomas, was noted in males at all doses and in females at the high dose. Statistical significance in pairwise comparisons was evident by the mid dose in males. The increase occurred among unscheduled deaths and terminal sacrifices, but not among the interim sacrifices (where the incidence was zero), suggesting that the tumors developed during the second year of the study (recognizing, of course, that the low number of interim sacrifices may preclude positive observations). In fact, no hemangiosarcoma or hemangioma was detected before week 72. It was thus assumed that it took at least one year for these lesions to develop to the point of detection. The animals that died prior to one year were not considered to be "at risk" and were not included in the potency calculations. The final total incidence rate in males (*i.e.*, the total number of tumor-bearing animals, understanding that multiple valscular neoplasms were present in some animals) was 2/66, 6/66, 10/69* and 10/68* at increasing doses (*p<0.05; see footnote #4, Table III-7c). The total number of vascular neoplasms (recognizing that more than one of such tumors was present in several animals) was 2/66, 9/66*, 13/69** and 18/68*** (*,**,***; p<0.05, 0.01, 0.0001, respectively) . In females the "at risk" incidence rate was 3/63, 3/70, 4/66 and 9/61. The hemangiosarcomas / hemangiomas were primarily localized to the liver, spleen and sternum, though other organ systems showed evidence of the tumors. Dose responsiveness through the whole dose range was apparent in the male liver only (0/66, 4/66, 5/69* and 7/68**). As explained in the report (p. 44), "nearly all of the vascular neoplasms were multicentric in origin, which is typical for this tumor type in CD-1 mice. The vascular system was therefore considered as a single tissue. Whether an animal had a vascular tumor in a single site or in multiple sites, it was counted as having only one vascular neoplasm... and entered under the organ *vascular tissue*".

Kidney: The incidence of renal tubular neoplasms increased markedly among the high dose males (unscheduled death and terminal sacrifices). Both carcinomas and adenomas were detected. The carcinomas were described as exhibiting "solid and trabecular / vascular patterns and were comprised of moderately differentiated renal tubular epithelium. Individual cells exhibiting nuclear karyomegaly and atypia were present. There was no evidence of metastasis" (p. 46). The adenomas "exhibited a solid growth pattern and were comprised of well-differentiated cells with only slight nuclear atypia" (p. 46).

Liver: Hepatocellular adenomas and carcinomas were notably increased among high dose females. These lesions were "comprised of moderate to well-differentiated hepatocytes and were morphologically compatible with the typical hepatocellular tumors commonly seen in aged CD-1 mice. There was no evidence of metastasis" (p. 47). Also, a hepatoblastoma was detected in one high dose female, though its relation to exposure was unclear.

The NOEL for non-oncogenic effects in this study was set at the low dose of 100 ppm (14.73 mg/kg/day in males, 18.11 mg/kg/day in females), based on the presence of intracytoplasmic droplets / pigment in the bladders of both males and females at the mid and high doses, and the inhibition of brain and RBC cholinesterases, also at the mid and high doses. It is also noted that there was an increased incidence of hemangiosarcomas at all doses (including the low and mid dose in males and the high dose only in females). However, the high dose exceeded the maximum tolerated dose based on the marked decrements in body weight changes noted throughout the study, female deaths noted in the first 7 weeks, and clinical signs. Even so, the appearance of hemangiosarcomas and hemangiomas in males at the other doses supports the conclusion that carbaryl is carcinogenic to mice. This study was considered acceptable by FIFRA guidelines.

Table III-7a. Effects of carbaryl on organ weights; 2 year CD-1 mouse study (Hamada, 1993b)

	Carbaryl dose, males (ppm) [a]				Carbaryl dose, females (ppm) [a]			
	0	**100**	**1000**	**8000**	**0**	**100**	**1000**	**8000**
Body wts. (g)								
Week 53	39.6	40.9	37.1	36.0*	31.7	35.4*	35.6*	32.1
Week 105	37.3	37.6	38.0	32.5*	32.4	34.0	32.6	27.6*
Brain (g) (wk. 105)	0.49	0.50	0.48	0.50	0.51	0.51	0.52	0.48
Lung (wk. 105)								
Absolute (g)	0.27	0.28	0.30	0.25	0.38	0.30	0.24	0.22*
Relative to:								
body wt. (%)	0.714	0.735	0.817	0.758	1.200	0.933	0.776	0.783
brain wt. (ratio)	0.546	0.564	0.608	0.508	0.753	0.578	0.465*	0.461*
Liver/gall bladder (wk 105)								
Absolute (g)	2.05	2.26	2.14	2.51	2.05	2.05	1.81	2.09
Relative to:								
body wt. (%)	5.434	5.905	5.735	7.522*	6.258	6.332	5.740	7.338*
brain wt. (ratio)	4.156	4.585	4.436	5.059	4.050	4.025	3.483	4.324
Kidney (wk 105)								
Absolute (g)	0.77	0.77	0.80	0.62	0.49	0.50	0.54	0.50
Relative to:								
body wt. (%)	2.038	2.016	2.143	2.466*	1.515	1.571	1.702	1.761*
brain wt. (ratio)	1.562	1.555	1.661	1.651	0.982	0.991	1.030	1.038
Ovary (wk 53)								
Absolute (g)	n/a	n/a	n/a	n/a	0.042	0.034	0.049	0.028*
Relative to:								
body wt. (%)					0.1324	0.0955	0.1373	0.0865*
brain wt. (ratio)					0.0818	0.0633	0.0918	0.0546*

* $p<0.05$

[a] Mean systemic doses: 0, 14.73, 145.99 and 1248.93 mg/kg/day in males and 0, 18.11, 180.86 and 1440.62 mg/kg/day in females

Table III-7b. Non-neoplastic changes in CD-1 mice exposed over a 2-yr period to dietary carbaryl (Hamada, 1993b)

		Carbaryl dose, males (ppm) [a]				Carbaryl dose, females (ppm) [a]			
		0	**100**	**1000**	**8000**	**0**	**100**	**1000**	**8000**
Bladder									
intracytoplasmic	I [b]	0/10^{+++}	0/10	6/10**	10/10***	0/10^{+++}	0/10	0/10	10/10***
droplets / pigment	U [b]	0/33^{+++}	0/39	4/31*	18/40***	0/34^{+++}	0/37	6/35*	19/35***
	T [b]	0/36^{+++}	0/31	9/37**	19/30***	0/34^{+++}	0/30	4/32*	25/32***
TOTAL [c]		**0/69^{+++}**	**0/70**	**13/68*** **	**17/70*** **	**0/68^{+++}**	**0/67**	**10/67*** **	**44/67*** **
Eye									
cataract, bilateral	U	0/33	1/39	2/33	3/40	1/36	1/39	1/38	2/38
	T	8/37^{++}	6/31	5/37	12/30	5/34 $^{+++}$	6/31	3/32	16/32**
cataract, unilateral	U	3/33^{+}	3/39	1/33	7/40	5/36	3/39	3/38	6/38
	T	5/37	5/31	10/37	7/30	10/34	1/31	8/32	7/32
TOTAL [c]		**16/70^{++}**	**15/70**	**18/70**	**29/70***	**21/70^{+++}**	**12/70**	**15/70**	**31/70**
Spleen									
pigment	I	0/10^{+++}	1/10	1/3	9/10***	1/10^{+++}	1/10	2/9	8/10**
extramedullary hematopoiesis	I	7/10	7/10	7/10	10/10^{+}	7/10	7/10	7/10	9/10
Intestine									
amyloidosis	U	7/26^{+}	8/34	12/29	16/36	10/33	7/38	4/32	12/37
(duodenum)	T	2/37	6/31	0/37	0/30	3/34	4/31	2/32	1/32
amyloidosis (colon)	U	0/32	0/39	0/32	1/39	0/34^{++}	0/38	0/37	3/38
	T	0/37	0/31	0/37	0/30	0/34	0/31	0/32	0/32
Testis									
amyloidosis	U	6/33^{++}	4/39	5/32	13/40	n/a	n/a	n/a	n/a
	T	0/37	1/31	0/37	1/30				
Gallbladder									
inflammation,	I	0/10	1/9	0/9	2/10	2/10	0/9	1/10	0/9
subacute	U	0/23	1/24	0/21	0/24	3/21	2/27	1/26	0/29
	T	1/37	3/30	4/36	4/30	1/34	5/30	6/32	5/31

*,**,***: p<0.05, 0.01, 0.001 (Fisher Exact Test) - tests performed by risk assessor.
$^{+}$,$^{++}$,$^{+++}$: p<0.05, 0.01, 0.001 (Cochran-Armitage Trend Test) - tests performed by risk assessor.

[a] Mean systemic doses: 0, 14.73, 145.99 and 1248.93 mg/kg/day in males and 0, 18.11, 180.86 and 1440.62 mg/kg/day in females.
[b] T, terminal sacrifice; U, unscheduled deaths; I, interim sacrifice
[c] Totals exclude the interim sacrifices.

Table III-7c. Neoplastic changes in CD-1 mice exposed over a 2-yr period to dietary carbaryl (Hamada, 1993b)

		Carbaryl dose, males (ppm) [a]				Carbaryl dose, females (ppm) [a]			
		0	**100**	**1000**	**8000**	**0**	**100**	**1000**	**8000**
Vascular tissue									
hemangiosarcoma (M)	I [b]	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
hemangioma (B)		0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
hemangiosarcoma (M)	U [b]	0/33	3/39	3/33	3/40	1/36^{++}	3/39	1/38	8/38*
hemangioma (B)		0/33	0/39	0/33	1/40	0/36	0/39	1/38	0/38
hemangiosarcoma (M)	T [b]	2/37	2/31	6/37	4/30	1/34	0/31	2/32	1/32
hemangioma (B)		0/37	1/31	1/37	2/30	1/34	0/31	0/32	0/32
TOTAL [c]		**2/70**	**6/70**	**10/70***	**10/70***	**3/70^{++}**	**3/70**	**4/70**	**9/70**
"at risk" total (♂ only) [d]		**2/66**	**6/66**	**10/69***	**10/68***	**3/63^{++}**	**3/70**	**4/66**	**9/61** [j]
Kidney									
tubule cell adenoma (B)	I	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
tubule cell carcinoma (M)		0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
tubule cell adenoma (B)	U	0/32	0/39	0/33	1/40	0/35	0/39	0/37	0/38
tubule cell carcinoma (M)		0/32	0/39	0/33	0/40	0/35	0/39	0/37	0/38
tubule cell adenoma (B)	T	0/37^{++}	0/31	0/37	2/30 [e]	0/34	0/31	0/32	0/32
tubule cell carcinoma (M)		0/37^{+++}	0/31	0/37	3/30	0/34	0/31	0/32	0/32
TOTAL [c]		**0/69^{+++}**	**0/70**	**0/70**	**6/70***	**0/69**	**0/70**	**0/69**	**0/70**
Liver									
hepatocellular adenoma (B)	I	1/10 [f]	0/10	1/10	0/10	0/10	0/10	0/10	0/10
hepatocellular carcinoma (M)		0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
hepatocellular adenoma (B)	U	3/32	1/39	4/33	2/40 [6]	0/35	0/39	0/37	1/38
hepatocellular carcinoma (M)		3/32	5/39	1/33	5/40	0/35	1/39	1/37	2/38
hepatocellular adenoma (B)	T	8/37	6/31	8/37 [g]	6/30 [g]	0/34^{+++}	0/31	1/32	6/32 [6]**
hepatocellular carcinoma (M)		3/37	2/31	2/37	3/30	1/34	0/31	0/32	1/32 [h]
TOTAL [i]		**17/79**	**14/80**	**15/80**	**16/80**	**1/79^{+++}**	**1/80**	**2/79**	**10/80****

*,**: p<0.05, 0.01 (Fisher Exact Test) - tests performed by risk assessor.
$^{+}$,$^{++}$,$^{+++}$: p<0.05, 0.01, 0.001 (Cochran-Armitage Trend Test) - tests performed by risk assessor.

[a] Mean systemic doses: 0, 14.73, 145.99 and 1248.93 mg/kg/day in males and 0, 18.11, 180.86 and 1440.62 mg/kg/day in females.
[b] T, terminal sacrifice; U, unscheduled deaths; I, interim sacrifice
[c] Totals exclude the interim sacrifices.
[d] Animals dying before 53 weeks were not considered to be at risk for harboring hemangiosarcomas or hemangiomas (the time of the first male unscheduled death in which a hemangiosarcoma / hemangioma was detected was week 72). There were 4, 4, 1 and 2 pre-week 53 deaths among males. These values were subtracted from the total number of animals to produce the number of animals considered to be "at risk".
[e] Includes one animal with multiple kidney tubule cell adenomas.
[f] Includes one animal with multiple hepatocellular adenomas.
[g] Includes two animals each at the mid and high doses with multiple hepatocellular adenomas.
[h] This animal exhibited multiple hepatocellular carcinomas.
[i] Because of the appearance of hepatocellular adenomas in the male interim sacrifices, all animals were considered to be "at risk".
[j] Hemangiosarcoma + hemangioma incidence in high dose females did not reach statistical significance with a Fisher Exact test. However, the p value was 0.056.

In a corollary study, Debruyne (1998) attempted to determine if carbaryl-exposed tissues from the mouse study of Hamada (1993b) were in a state of heightened cell proliferation after one year of exposure. Proliferative state was assessed by the extent of immunohistochemical staining for proliferating cell nuclear antigen (PCNA). Based on its high proliferative state, a section of rat duodenum served as the positive control. Deparaffinized female liver and male kidney sections from the 8000 ppm mice (10/group, sacrificed after 52 weeks of exposure) were compared with parallel sections from control animals. The tissues were reacted with PCNA, amplified with a secondary antibody, exposed to streptavidin-peroxydase and further reacted with the chromogen aminoethylcarbazol. PCNA-positive (proliferating) cells had red-stained nuclei while non-proliferating nuclei were blue. 1000 cells were evaluated per section of liver and kidney. For male kidneys, PCNA-positive renal cortical tubular cells had a mean of 1.20±1.75 per 1000 cells (range of 0 to 4), while treated tissue had 3.90±2.18 (range of 1 to 7). For female hepatocytes, the control mean was 4.60±7.68 (range of 0 to 23) and treated 8.33±3.84 (range of 2 to 13). The results were interpreted as of uncertain toxicologic significance for male kidneys and not significant for female livers, based (1) on the range of variability and the small difference in males and (2) on the observation that all treated female values were within the control range. Thus increased cell cycling of putative target cells was not clearly demonstrated. The positive control data from the rat were not, however, included in the report.

This study was considered to be supplemental.

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The following two studies were designed to determine if carbaryl's vascular tumorigenic effect in mice is mediated through a process involving the p53 tumor suppressor gene.

Bigot (1999) attempted to validate the p53 knockout mouse system as a rapid predictor of rodent geno-carcinogenicity, particularly with respect to vascular tumors in mice. Male mice, strain C57B1/6 Tac-[KO]Trp53N5-T, heterozygous for the p53 tumor suppressor gene, were compared with wild type male mice for response to urethane, a genotoxic compound known to induce vascular tumors in lifetime studies in mice, and to d-limonene, which is not considered to be carcinogenic in mice. 20 mice per dose group were treated with 0, 1, 10 or 100 mg/kg/day of urethane by gavage for at least 180 days, or with d-limonene at 250 mg/kg/day. Wild type mice were given vehicle only. Body weights, food consumption and clinical signs were recorded. At necropsy, all major organs were examined, selected organs weighed, and tissues prepared for histopathology.

At 100 mg/kg/day urethane, only 3 animals survived to termination. Two animals died at 10 mg/kg/day. A total of 18/20 mice at 100 mg/kg/day urethane had vascular neoplasms, which were predominantly in the liver, at 181-184 days. At 10 mg/kg/day, 1/20 had a vascular tumor. No such tumors were observed at 1 mg/kg/day or among controls. D-limonene exposure resulted in hyperplasia of the non-glandular stomach, but was negative for tumor induction. These results supported the p53 knockout mouse as a model for identifying vascular tumors induced by genotoxic carcinogens.

This study is considered to be supplemental.

Chuzel (1999) used the p53 knockout mouse system, which was validated above for its rapid sensitivity to genotoxic carcinogens, particularly in the vascular system, to test whether carbaryl can act to produce such tumors within a six month period. The first male unscheduled death showing a hemangioma / hemangiosarcoma in the study of Hamada (1993b) was detected in

week 72. Consequently, the study was carried out to see if such tumors appeared in the p53 knockout mouse before that time, which would suggest that carbaryl acted in a similar, presumably genotoxic, manner as urethane (Bigot, 1999).

Carbaryl (99% purity) was fed in the diet to groups of 20 male mice for at least 180 days. Mice were C57B1/6 Tac-[KO]Trp53N5-T, heterozygous for the p53 tumor suppressor gene. Doses were 0, 10, 30, 100, 300, 1000 or 4000 ppm, resulting in mean achieved doses of 0, 1.76, 5.21, 17.5, 51.6, 164.5 and 716.6 mg/kg/day body weight, food consumption and clinical signs were recorded. Selected organs were weighed and tissues prepared for histopathologic examination. All control and high dose animals were examined, as were all decedents. No treatment-related deaths were reported. There were some effects on body weight and food consumption at 1000 and 4000 ppm. The major non-neoplastic finding was the presence of an accumulation of "globular deposits" in the umbrella cell layer of the urinary bladder. The total incidence was 0/20, 0/20, 0/20, 11/20, 20/20, 20/20 and 20/20 at ascending doses. The appearance was transparent, slightly yellow and birefringent at 100, 300 and 1000 ppm, and smaller but with a red-brown color at 4000 ppm.The severity of the accumulation increased with dose. There was no reported local irritation or hypertrophy of the bladder epithelium. Relative organ weights were increased in heart, liver and kidney at 4000 ppm and for kidney at 1000 ppm as well.

The NOEL was set at 30 ppm (5.2 mg/kg/day) based on the histopathologic observations ("globular deposits" in the umbrella cell layer of the urinary bladder) at 100 ppm. There was no treatment-related evidence of neoplasia or preneoplasia in vascular tissue or any organs examined. Several spontaneous neoplasms were found, though none were present at 4000 ppm. The negative result in this study lowered the possibility that carbaryl-induced neoplasms in male CD-1 mice, including hemangioma / hemangiosarcoma, resulted from processes mediated by the p53 tumor suppressor gene in a manner similar to urethane. However, genotoxicity could not be totally excluded as a mode of action for carbaryl.

This study was considered to be supplemental.

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The following two open literature studies examine the question of whether carbaryl can modulate tumor production in mice in the context of stimulation by other carcinogens. The first, by Triolo *et al.* (1982), shows an increase in the number of lung tumors when two gavage treatments with benzo[*a*]pyrene were accompanied by 20 weeks of exposure to dietary carbaryl. The second, by Shukla *et al.* (1992), shows that carbaryl has skin tumor initiating capability in a standard initiation-promotion protocol using phorbol ester as the promoter. Taken together, the studies emphasize that data from standard rodent oncogenicity studies do not provide a complete picture of carbaryl's oncogenic effects.

Triolo *et al.* (1982) studied the effects of dietary carbaryl on benzo[*a*]pyrene (BP)-induced lung tumor production (dietary toxaphene was also examined, but will not be discussed here). Female A/J mice, 11-31 animals per treatment, received feed containing 0 (5% corn oil) or 1000 ppm carbaryl for 20 weeks, with the choice of dose based on preliminary data indicating no effect on body weight gain. Three mg BP was administered by intubation on study days 7 and 21. After the 20-wk period, the animals were sacrificed for tumor enumeration (only tumors greater than 1 mm were counted). Similarly treated mice were analyzed for liver and lung BP hydrolase (BPH), an enzyme involved in the metabolism of BP.

Carbaryl had neither a convincing nor a consistent effect on lung tumor incidence in the absence of BP. For example, the insecticide was associated with an increase in the percentage of mice with tumors in one experiment (9% to 31%, not statistically significant, Expt. #1) and a

decrease in another experiment (23% to 10%, Expt. #2). The number of tumors per mouse increased slightly in both experiments, though in non-statistically significant manner (Expt. #1 from 1.0±0.0 to 1.2±2.2; Expt. #2 from 1.1±0.0 to 1.3±0.3). However, in the only experiment conducted in the presence of BP, carbaryl increased the percentage of mice with tumors from 88% to 100% (not statistically significant) and the number of tumors per mouse from 3.7±0.6 to 5.7±1.4 ($p<0.05$). Assay of BPH activity in non-BP-treated animals showed no statistically significant effects in liver or lung. In the presence of BP, a statistically significant increase in BPH activity, from 3.06±0.14 pm/mg protein to 3.86±0.11 pm/mg protein ($p<0.025$), was noted in the lung, but not in the liver. The authors speculated that the increased lung enzyme activity may be mechanistically related to the increased tumor production in the same organ, though this will require further experimental verification.

Shukla *et al.* (1992) examined the ability of carbaryl to act as a complete carcinogen, initiator and / or promoter following dermal exposure in female Swiss albino mice (20 per dose group). Each experiment ran for ~52 weeks (promotion treatment continued for 51 weeks after initiation).

Expt. #1 (complete carcinogenesis): Group I - untreated controls; Group II - 5 µg benzo[*a*]pyrene (BP), 3x/wk; Group III - 100 mg/kg carbaryl, 3x/wk; Group IV - vehicle control, 100 µl acetone, 3x/wk. *Result*: tumors were identified only in Group II (100% of survivors formed skin tumors by the end of the study).

Expt. #2 (initiation): Group I - untreated controls; Group II - single treatment with 100 mg/kg carbaryl, followed 1 week later by 5 µg 12-O-tetradecanoyl phorbol-13-acetate (TPA), 3x/wk; Group III - multiple treatments (3x/wk for 3 weeks) with 100 mg/kg carbaryl, followed 1 week later by 5 µg TPA, 3x/wk; Group IV - single treatment with 52 µg DMBA, followed 1 week later by 5 µg TPA, 3x/wk; Group V - multiple treatments (3x/wk for 3 weeks) with 100 mg/kg carbaryl, followed 1 week later by 100 µl acetone, 3x/wk; Group VI - multiple treatments(3x/wk for 3 weeks) with 100 µl acetone, followed 1 week later by 5 µg TPA, 3x/wk. *Result*: 2/17 survivors from Group II (single carbaryl treatment initiation protocol), 8/13 survivors from Group III (multiple carbaryl treatment initiation protocol), and 16/16 survivors from Group IV (single DMBA treatment initiation protocol) showed skin tumors; no other group showed tumors.

Expt. #3 (promotion): Group I - untreated controls; Group II - single treatment with 52 µg DMBA, followed 1 week later by 100 mg/kg carbaryl, 3x/wk; Group III - single treatment with 52 µg DMBA, followed 1 week later by 5 µg TPA, 3x/wk; Group IV - single treatment with 100 µl acetone, followed 1 week later by 100 mg/kg carbaryl, 3x/wk; Group V - single treatment with 52 µg DMBA, followed 1 week later by 100 µl acetone, 3x/wk. *Result*: only Group III (DMBA initiation, TPA promotion) resulted in tumors (16/16 survivors).

These results indicate that while carbaryl was negative for complete carcinogenesis and for promotion, it does indeed act as an initiator in the mouse 2-stage skin carcinogenesis protocol. All tumors were considered benign in nature (pedunculated and flat squamous cell papillomas, flat squamous cell papillomas, keratoacanthomas and mixed type tumors).

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Dogs. Hamada (1987) exposed 6 beagles/sex/dose group to carbaryl (purity, 99%) in the diet for one year. The doses were 0, 125, 400 and 1250 ppm, corresponding to average systemic doses of 0, 3.4, 11.2 and 33.8 mg/kg/day in males and 0, 3.7, 11.0 and 34.4 mg/kg/day in females. Twice daily observations were made for mortality / moribundity, once daily for clinical signs. Body weight and food consumption were determined weekly, plasma and RBC cholinesterase activities three times prior to treatment (weeks -3, -2 and -1) and during weeks 5, 13, 26 and 52. Brain cholinesterase activity was determined at study termination. Additional

laboratory studies measured conventional hematologic, clinical chemical and urinalysis parameters at weeks -2, 13, 26 and 52. Ophthalmologic exams were conducted before the initiation of treatment and at termination. Necropsies, organ weight determinations and histopathology were also executed at termination.

There were neither deaths nor clinical signs attributable to carbaryl during the study. Nonetheless, high dose females gained less weight than controls throughout. This decrement achieved statistical significance between weeks 0-5 only (female weight gain, ascending doses, weeks 0-5: 1.0, 1.1, 1.0, 0.5* kg; *$p<0.05$), consistent with slightly lowered food consumption for each period (not statistically significant). There were statistically significant increases in white blood cell counts among high dose males at week 26 (10.3, 10.7, 10.4, 13.4* Th/µl) and 52 (10.3, 11.1, 9.9, 15.2* Th/µl), and in segmented neutrophil counts at week 52 (7.0, 8.2, 7.5, 11.4* Th/µl). High dose females showed statistical decrements in albumin levels at all measurement intervals (*eg.*, at week 52: 3.5, 3.5, 3.4, 3.2* g/dl).

Cholinesterase activities were suppressed at all time points, often by statistically significant margins (Table III-8). For brain cholinesterase, the level of inhibition reached 36% at the high dose, though even at the low dose a statistically significant 20% level of inhibition was noted in females. RBC cholinesterase inhibition as high as 56% was noted at the high dose (week 5), with non-statistically significant inhibition as high as 14% (week 13) noted at the low dose. Plasma cholinesterase inhibition reached 66% (week 5) at the high dose, with statistically significant inhibition as high as 23% (week 13) noted at the low dose.

Gross necropsies were unremarkable. Organ weight determinations revealed a significant increase in absolute liver weight in high dose males (242, 255, 269, 301* g; *$p<0.05$), though a corresponding effect was not evident in females. A statistically significant decrement in thyroid weight relative to body weight was also noted in males (0.011, 0.009, 0.010, 0.008*), but was not accorded biological significance. Histopathology did not reveal lesions that were clearly dependent on carbaryl exposure.

The LOEL was set at 125 ppm (3.4-3.7 mg/kg/day), based on cholinesterase inhibition (brain, RBC and plasma). Because this was the low dose, a corresponding NOEL was not set. This study was deemed acceptable by FIFRA standards.

Table III-8. Suppression of cholinesterase activities in beagle dogs by dietary carbaryl; 1-yr study (Hamada, 1987)

Time	Carbaryl dose (ppm), males [a]				Carbaryl dose (ppm), females [a]			
	0	125	400	1250	0	125	400	1250
Plasma ChE, µmol/ml								
Week -1	8.5±2.02	8.5±1.02	8.1±1.58	7.8±1.50	8.8±1.04	7.4±1.02	8.9±0.96	9.0±1.40
Week 5	8.5±1.83	7.3±1.04 86% [b]	5.4±1.12* 64%	2.9±0.84* 34%	8.1±1.47	6.3±0.73* 78%	5.6±0.82* 69%	3.2±0.81* 40%
Week 13	8.6±1.94	7.5±1.16 87%	5.7±1.07* 66%	3.7±0.99* 43%	8.6±0.91	6.6±0.77* 77%	6.2±1.15* 72%	3.7±0.71* 43%
Week 26	8.6±1.98	7.4±1.05 86%	5.6±1.02* 65%	3.5±1.00* 41%	8.9±1.02	7.2±1.48* 81%	6.6±1.04* 74%	4.0±0.92* 45%
Week 52	8.1±2.49	7.8±1.31 96%	5.7±1.21* 70%	3.4±1.14* 42%	7.7±1.24	6.8±1.20 88%	7.0±1.71 91%	4.1±1.08* 53%
RBC ChE, µmol/ml								
Week -1	7.6±1.66	7.4±1.45	7.5±1.59	5.6±0.81	9.4±2.17	9.3±1.23	8.9±1.03	10.0±0.94
Week 5	7.3±1.42	6.5±1.23 89%	5.6±0.90* 77%	3.20±0.73* 44%	10.5±1.99	9.1±1.55 87%	6.9±0.76* 66%	6.5±0.79* 62%
Week 13	7.2±1.43	6.2±1.46 86%	5.2±0.61* 72%	3.7±0.84* 51%	8.6±1.85	8.3±1.66 97%	6.1±0.68* 71%	6.1±0.76* 71%
Week 26	8.0±1.21	7.5±1.18 94%	6.5±0.94 81%	4.3±0.87* 54%	10.4±1.66	9.5±1.24 91%	7.4±0.95* 71%	6.6±0.99* 63%
Week 52	8.5±1.77	7.9±1.50 93%	6.8±0.89 80%	4.0±0.55* 47%	10.0±2.03	9.3±1.26 93%	8.2±1.09 82%	7.0±0.64* 70%
Brain ChE, µmol/g								
Week 52	11.3±3.41	9.7±2.90 86%	7.7±2.07 68%	8.5±1.38 75%	9.0±1.23	7.2±0.64* 80%	7.0±1.19* 78%	5.8±0.48* 64%

*p<0.05

[a] Equivalent to average systemic doses of 0, 3.4, 11.2 and 33.8 mg/kg/day in males and 0, 3.7, 11.0 and 34.4 mg/kg/day in females.

[b] Percent of concurrent control activities.

Table III-9. NOEL and LOEL values for subchronic and chronic toxicity studies on carbaryl

Species, strain	Study type & exposure regimen	Effects at LOEL	NOEL	LOEL	Reference [f]
Subchronic studies:					
Dog, Beagle	5-wk dietary	none	125 ppm[a] (M: 3.83 mg/kg/day; F: 4.11 mg/kg/day)	>125 ppm[1] (M: 3.83 mg/kg/day; F: 4.11 mg/kg/day)	*Supplemental* Hamada (1991)
Mouse, TSG p53 wild type	4-wk dietary, males only	⇑relative liver wt. [b]	1000 ppm (222 mg/kg/day)	2000 ppm (424 mg/kg/day)	*Supplemental* Dange (1998)
Rat, Wistar	50-day dietary, males only	⇓ maze function, ⇓ brain ChE activity, altered EEG patterns	<10 mg/kg/day	10 mg/kg/day	*Supplemental* Desi *et al.* (1974)
Rat, Sprague-Dawley	4-wk dermal [c]	systemic: no systemic toxicity local: atonia ChE: inhibition of brain & RBC ChE	systemic: 100 mg/kg/day[a] local: 50 mg/kg/day ChE: 20 mg/kg/day	systemic: >100 mg/kg/day[a] local: 100 mg/kg/day ChE: 50 mg/kg/day	*Supplemental* Austin (2002a)
Chronic studies:					
Dog, Beagle	1-yr dietary [e]	⇓ brain, RBC and plasma ChE activity	**<125 ppm (3.4-4.7 mg/kg/day)**	125 ppm (3.4-4.7 mg/kg/day)	*Acceptable* Hamada (1987)
Mouse, CD-1	2-yr dietary	bladder histopathologic effects, inhibition of brain & RBC ChE [d]	100 ppm (~14.73 mg/kg/day)	1000 ppm (~145.99 mg/kg/day)	*Acceptable* Hamada (1993b)
p53 Mouse, CD-1 (C57B1/6 Tac-[KO]Trp53 N5-T)	6-month dietary	globular deposits in the umbrella cell layer of the urinary bladder	30 ppm (~5.2 mg/kg/day)	100 ppm (17.5 ppm)	*Supplemental* Chuzel (1999)
Rat, Sprague-Dawley	2-yr dietary	⇓ wt. gain and ⇓ brain ChE activity	250 ppm (10.0-12.6 mg/kg/day)	1500 ppm (60.2-78.6 mg/kg/day)	*Acceptable* Hamada (1993a);

[a] Highest dose tested.
[b] This LOEL determinant was considered conditional - there was no histopathology done to determine if the increased relative liver weight was adverse in nature.
[c] Dermal exposure was for 5 days/wk, 6 hr/day.
[d] There was also an increase in hemangiosarcomas at all doses in the Hamada (1993b) mouse study.
[e] The 1-yr dog dietary study represents the critical chronic study. Benchmark dose calculations indicate an LED_{10} of 0.5 mg/kg/day.
[f] The designation "Acceptable" indicates that the study was successfully completed according to FIFRA guidelines. "Supplemental" indicates that the study was not conducted according to FIFRA guidelines; however, such studies were reviewed and considered to contribute to the general genotoxic picture of the chemical.

E. GENOTOXICITY

1. Overview

Carbaryl failed to produce gene mutations in four of the five *in vitro* studies reviewed, including two FIFRA-compliant studies and two supplemental studies. Carbaryl did produce mutations to ouabain resistance in one supplemental study in V79 Chinese hamster fibroblasts. Carbaryl also caused chromosomal aberrations in four of six studies, including one FIFRA-compliant study. Of those four positive studies, only one was *in vivo*, and that was in *Allium cepa* (onion tree), which was of questionable relevance to mammalian systems. Both of the negative chromosomal aberration studies (one FIFRA-compliant, one supplemental) were performed *in vivo*. Two of the four reviewed DNA damage studies were positive. Both of these studies were supplemental and both were performed *in vitro*. The two negative studies, one *in vivo* and one *in vitro*, were FIFRA-compliant.

One reviewed study demonstrated that nitrosocarbaryl could be produced from carbaryl and nitrite under acidic *in vitro* conditions. A separate study showed that nitrosocarbaryl caused chromosomal aberrations in Chinese hamster fibroblasts. Finally, one study in V79 Chinese hamster fibroblasts showed that, like carbaryl, the carbaryl metabolite ∝-naphthol was toxic and induced c-mitosis, an aberrant form of mitosis that may reflect effects on mitotic spindle formation.

The results of the genotoxicity tests are summarized in Table III-10.

2. Gene mutation

Lawlor (1989) studied the potential mutagenicity of carbaryl (99.3% purity) in the Ames *Salmonella* reverse mutation assay. The tester strains were TA1535, TA1537, TA1538, TA98 and TA100. Two trials were conducted, with and without Aroclor 1254-induced male Sprague-Dawley rat liver microsomes, using triplicate platings at a range of doses. A preliminary test indicated cytotoxicity at and above 667 µg/plate. Use of positive control substances indicated the appropriate sensitivity of the tester strains. Trial 1: 0 (DMSO), 5, 10, 50, 100, 500, 1000 µg/plate; Trial 2: 0 (DMSO), 10, 50, 100, 500, 1000, 2000 µg/plate. There was no evidence for an increased reversion rate at any carbaryl concentration. Carbaryl was not considered to be mutagenic in this assay.

The study was considered acceptable according to FIFRA guidelines.

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Young (1989) studied the potential mutagenicity of carbaryl (99.3% purity) in the Chinese hamster ovary cell / HGPRT forward mutation assay both with and without an Aroclor 1254-induced rat liver microsomal activation system. There were two trials, each using a single culture per concentration. Without activation, trial 1: 0 (DMSO), 0.001, 0.01, 0.03, 0.05, 0.08, 0.1, 0.15, 0.2, 0.3 (toxic) mg/ml; trial 2: 0 (DMSO), 0.01, 0.05, 0.1, 0.15, 0.2, 0.25 (toxic), 0.3 (toxic) mg/ml. With activation, trial 1: 0 (DMSO), 0.001, 0.01, 0.03, 0.05, 0.08, 0.1, 0.15, 0.2, 0.3 (toxic) mg/ml; in trial 2, only one concentration could be scored due to cytotoxicity with a new lot of S9 microsomes; trial 3: 0 (DMSO), 0.001, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 (toxic), 0.13 (toxic) mg/ml. There was no reproducible increase in forward mutations. Carbaryl was not considered to be mutagenic in this assay.

This study was considered acceptable according to FIFRA guidelines.

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Ahmed *et al.* (1977a) studied the ability of carbaryl and three other pesticides (chlordane, dieldrin and 2,4-D-fluid) to induce ouabain-resitant mutants in V79 Chinese hamster cells. Cell

survival in the presence of 1 µM ouabain was determined using a concentration range of 0.1 - 1000 µM. For the mutation assay, thirty 9-cm plates were seeded with 10^5 cells/plate, followed 4 hours later by the addition of 10 µM pesticide (calculated to generate survival of 40-75%). After 48 hours, 1 mM ouabain was added. Resistant colonies were enumerated 1 week later.

Under these conditions, carbaryl, chlordane, dieldrin and 2,4-D-fluid induced mutation frequencies (per 10^4 survivors) of 15.3, 26.9, 16.4 and 25.5, respectively (control frequency: 1.8). Percent survival was 66, 44.4, 77.8 and 41.3 (controls: 93). Subculture of resistant colonies in the presence of ouabain showed that resistance was maintained, thus indicating that the change was heritable.

This study was considered to be supplemental.

3. Chromosomal aberrations

Weil (1972) studied the potential for carbaryl-induced dominant lethal mutations in rats (strain not identified) as part of a 3-generation reproductive toxicity, teratology and mutagenicity study. Animals were dosed 5 days/week with carbaryl (99.6% purity) in feed at 0, 7, 25,100 or 200 mg/kg/day, by gavage in corn oil at 0, 3, 7, 25 or 100 mg/kg/day, or in feed containing corn oil at 0 or 100 mg/kg/day. The F_{2a} males were withdrawn at 7 months old and mated weekly with unexposed females for 10 weeks. The feeding study (pure and with corn oil) evidenced no dose-related or consistent differences from controls, resulting in a NOEL of 200 mg/kg/day. The gavage study resulted in a significant reduction in mean implants or viable fetuses at 100 mg/kg/day for the week 8 matings only: 12.5 *vs.* 14.0 (controls) and 11.5 *vs.* 14.0 (controls), respectively. As this was not regarded as meaningful, the gavage NOEL was set at 100 mg/kg/day.

This study was deemed supplemental.

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Murli (1989) studied the potential for carbaryl-induced chromosomal aberrations *in vitro* using Chinese hamster ovary (CHO)-WBL cells. Duplicate cultures were exposed to carbaryl (99.3% purity), without activation, to 0 (negative and solvent), 7.5, 10, 25, 50 or 75 µg/ml for 17.5 hours incubation, with harvest at 20 hours. Further duplicates were exposed, with S9 microsomal activation, to 0 (negative and solvent), 150, 200, 250 or 300 µg/ml for 2 hr with harvest at 20 and 30 hours. In both cases, the positive controls were functional. The harvest times were based on a preliminary study with BrdUrd staining for determination of cell cycles. Cytotoxicity was evident at around 25 µg/ml in the absence and 200 µg/ml in the presence of S9 microsomes. No increase in aberrations was detected in the absence of an S9 activation system. However, in the presence of S9, an increase in aberrations/cell, percent cells with aberrations and percent cells with >1 aberration at both harvest times was detected at all doses. Dose responsiveness was not evident. Thus the lowest effective level of carbaryl was not determined. The types of aberrations seen included chromatid and chromosome gaps, chromatid and chromosome breaks, interstitial deletions, triradials, quadriradials, complex rearrangements and dicentrics. Carbaryl is considered to be active in this assay in the presence of an S9 activating system.

This study was considered acceptable by FIFRA guidelines.

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Marshall (1996) studied the potential for carbaryl to induce micronuclei *in vivo* in the bone marrow erythrocytes of treated CD-1 mice. Animals were dosed with carbaryl (99.9% purity) in 0.5% carboxymethylcellulose at doses of 0, 50, 100 or 200 mg/kg/day for two consecutive days. Five animals/sex/dose were sacrificed after a further 24 or 48 hours. Cyclophosphamide was

used as the positive control and was functional. At 200 mg/kg the animals showed lethargy, which lasted ~2 hours after the first dose, with eye closure in 3 females and eye secretions in one. Body weight loss was detected in 2 males and 10 females at the high dose. For each animal, 2000 polychromatic erythrocytes were scored for micronuclei and the PCE/NCE ratio was reported. Carbaryl did not induce micronuclei in this study.

This study was considered acceptable by FIFRA guidelines.

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Ishidate and Odashima (1977) screened 134 compounds (including carbaryl and nitrosocarbaryl) for their ability to produce chromosomal aberrations *in vitro* in a clonal subline of a Chinese hamster fibroblast line. Each chemical was tested at three doses, including the 50% growth inhibition dose. Following 6-48 hr of exposure, the cells were treated with colcemid, trypsinized, incubated in hypotonic KCl solution, fixed, a few drops of suspension applied to a clean slide, which was then dried and stained. One hundred well-spread metaphases were examined for chromatid gaps, chromatid breaks, chromatid or chromosomal translocations, ring formation, fragmentation / pulverization and polyploidy.

Sixty three of 134 compounds were negative (defined as less than 4.9% cells with aberrations), 17 / 134 were suspicious (5.0% - 9.9% with aberrations) and 54 / 134 were positive (> 10% positive cells). Carbaryl (referred to as naphthyl-*N*-methylcarbamate) generated 35.0% aberrations after 48 hours at a maximum effective dose of 0.03 mg/ml ($1.5x10^{-4}$ M). The types of aberrations included chromatid gaps (predominant), chromatid / chromosomal breaks (predominant), translocations (predominant), ring formation and fragmentation. Nitrosocarbaryl (referred to as naphthyl-*N*-nitroso-*N*-methylcarbamate) generated 81.0% aberrations after 24 hours at a maximum effective dose of 0.015 mg/kg ($0.7x10^{-4}$ M). The types of aberrations included chromatid gaps, chromatid / chromosomal breaks (predominant), translocations (predominant) and ring formation.

This study was considered to be supplemental.

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Soderpalm-Berndes and Onfelt (1988) studied the effects of carbaryl and ∝-naphthol on mitosis in V79 Chinese hamster fibroblasts. Of particular interest was the possible role of antioxidants (selenite and ∝-tocopherol) in modulating the toxic response. Parameters followed included (1) survival (measured as post treatment colonizing percentage following trypsinization); (2) c-mitosis (not precisely defined in the report, though presumably an aberrant mitotic form; measured after fixation of cells grown directly on microscope slides) and monopolar mitotic configurations (basically indicators of the location of the metaphase chromosomes in mitotic cells); (3) TCA-soluble sulfhydryls (*i.e.*, non-protein sulfhydryls - NPSH - considered to represent mostly reduced glutathione) and protein sulfhydryls (PSH - presumably an indication of the extent of macromolecular crosslinking); (4) various enzyme activities (glutathione-S-transferase, selenium-dependent glutathione peroxidase and glutathione disulfide reductase); (5) ATP measurements; and (6) lipid peroxidation (using the "thiobarbituric acid test").

(1) In the absence of antioxidants, carbaryl and ∝-naphthol were toxic (*i.e.*, inhibited colony formation upon subculture) in the 200-400 µM range. Addition of ∝-tocopherol ameliorated this response somewhat.

(2) Induction of c-mitosis in the absence of antioxidants passed the 10% point between 50 and 100 µM carbaryl, reaching 60-70% at 400 µM. In the presence of antioxidants the 10% point occurred between 100 and 200 µM, with c-mitosis reaching 37-42% at 400 µM carbaryl. ∝-naphthol was less efficient in inducing c-mitosis. In addition, the number of metaphase cells with a normal-shaped morphology and centrally located chromosomes decreased from nearly 100%

to less than 50% in the presence of 400 µM carbaryl, having been replaced by elongated cells with chromosomes located at one pole. The antioxidants appeared to have little effect on this process.

(3) Carbaryl treatment between 25 and 400 µM resulted in a dose-dependent decrease in NPSH (100% in controls, 88% at 25 µM, 32% at 400 µM) and, to a lesser extent, PSH (97% at 25 µM, 79% at 400 µM). Neither ∝-tocopherol nor selenite affected NPSH levels, though there may have been a ∝-tocopherol-based protective effect on PSH levels at 200 and 400 µM carbaryl. ∝-Naphthol had no effect on NPSH or PSH levels.

(4) ∝-Tocopherol treatment increased glutathione-S-transferase activity by 39% in the absence of carbaryl and by 13% in the presence of 400 µM carbaryl. Selenite increased selenium-dependent glutathione peroxidase activity by 50% in the absence of carbaryl and by 12% in the presence of 400 µM carbaryl.

(5) In the absence of glucose, both carbaryl and ∝-naphthol reduced the amount of cellular ATP, particularly at 200 and 400 µM. The investigators suspect that ∝-naphthol (produced from carbaryl metabolism) may be an uncoupler of oxidative phosphorylation.

(6) Lipid peroxidation was increased in a dose-dependent manner between 25 (~110% of controls) and 400 µM (~170% of controls) carbaryl. Selenite and ∝-tocopherol both greatly reduced the peroxidation.

The investigators concluded from these data that carbaryl affects mitotic spindle formation, possibly through a decrease (reversible and irreversible) in cellular levels of glutathione S-transferase activity or through an increase in lipid peroxidation (which may itself be a manifestation of lowered glutathione S-transferase activity). They discounted a role for glutathione S-transferase or lipid peroxidation in the formation of monopolar metaphase configurations.

This study was considered to be supplemental.

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Grover *et al.* (1989) examined the ability of carbaryl to induce mutations in the *Salmonella* histidine reversion assay and chormosomal aberrations in root meristems of *Allium cepa*.

Carbaryl was negative in the *Salmonella* assay (three strains tested: TA98, TA102 and TA1535) both in the absence and presence of S9 and S14 microsomes (the latter were derived from wheat seedlings). Doses tested were 0, 1, 5, 10, 50, 100, 500, 1000, 5000 and 10,000 µg/plate. Depending on the strain and the presence/absence of activating microsomes, toxicity became evident between 1000 and 5000 µg/plate.

Incubation of *A. cepa* root tips in solutions containing 0, 0.1, 0.4, 0.7, 1.0 or 1.3% carbaryl depressed the mitotic rate and induced mitotic and chromosomal abnormalities in the exposed cells both in the presence and absence of S14 microsomes, essentially at all doses. While the relevance of the root tip assay to mammalian cells *in vitro* or *in vivo* is not known, these results are consistent with other studies on carbaryl which indicate that it disturbs mitosis and chromosomal integrity.

This study was considered to be supplemental.

4. DNA damage

Cifone (1989) studied the potential for carbaryl to induce unscheduled DNA synthesis *in vitro* in primary hepatocytes isolated from adult male Fischer 344 rats. Cultures were exposed to carbaryl (99.3% purity) at 0 (DMSO), 0.5, 1, 2.5, 5, 10 or 25 µg/ml (trial 1), or 0 (DMSO), 5, 7.5, 10, 15, 20 or 25 µg/ml (trial 2). Higher concentrations were also tested, though the level of toxicity seen at those concentrations was inconsistent with a valid test. 150 cells per concentration from triplicate coverslips were scored. A small increase in labeled nuclei was

noted at 10 µg/ml in trial 1 (average % nuclei with ≥6 grains = 20.4, *vs.* 4.7 in solvent controls, 3.3-6.7% in the remaining doses [including 25 µg/ml], and 99.3% in the presence of 2-acetylaminofluorene [2-AAF]). However, the lack of a dose response, the non-reproducibility in trial 2, and the absence of an increase in net nuclear grains at any dose, make it unlikely that carbaryl was active in this assay. In conclusion, these data were not supportive of an inductive role for carbaryl in unscheduled DNA synthesis in this study.

This study was considered acceptable by FIFRA guidelines.

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Sagelsdorff (1994) studied the potential for hepatic protein- and DNA- binding by carbaryl in male CD-1 mice. Covalent binding of [1-^{14}C-naphthyl]--carbaryl to chromatin protein and to DNA was determined using 5 groups of 4 - 6 animals per group. Group 1: one dose of 75 mg/kg labeled carbaryl by gavage; Group 2: fed 8000 ppm carbaryl for 13 days prior to a single dose as for Group 1; Group 3: untreated (used for controls of the extraction procedures); Group 4: fed 8000 ppm carbaryl for 14 days; Group 5: untreated. Groups 4 and 5 were not processed. Urinary excretion was measured for a single animal from Groups 1 and 2 over 24 hours and found to be 33% and 31%, respectively, of the administered dose. Fecal excretion was not measured. Livers from 2 animals of the same group were pooled for processing - they were homogenized, chromatin precipitated, deproteinated and DNA further pruified on hydroxyapatite, dialyzed and precipitated with ethanol. Chromatin protein was precipitated with acetone and dissolved in 1% SDS several times. Radioactivity was determined by liquid scintillation counting. Binding was determined as a function of mg protein and mg DNA. The pmol/mg binding to protein ranged from 7 to 11, with no difference between groups 1 and 2. For DNA, binding (dpm/mg) was <5.99 with an 80% counting efficiency and a limit of detection of 2.7 cpm over background. The Covalent Binding Index (CBI) was calculated to be <0.1 as a maximum DNA-binding ability. This result gives no indication for a genotoxic potential for carbaryl mediated by DNA binding. The CBIs for strong hepatocarcinogens (*eg.*, aflatoxin B1) are orders of magnitude higher.

This study was deemed acceptable by FIFRA standards.

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Ahmed *et al.* (1977b) studied the ability of carbaryl and 12 other pesticides to induce unscheduled DNA synthesis (UDS) in SV-40 transformed human cells (VA-4) *in vitro*, both in the presence and absence of an S9 microsomal activating system. VA-4 cells can be propagated indefinitely in culture and exhibit similar DNA repair characteristics to the human primary fibroblast cultures, Detroit-500 and WI-38. Cells were seeded onto 11.22 mm coverslips in 100-mm dishes at $10^4/cm^2$. Twenty four hours later hydroxyurea was added to inhibit semiconservative DNA synthesis. ^{3}H-thymidine, hydroxyurea and the test article (±S9) were added after 5 further hours. Coverslips were removed at intervals of 1, 3, 5, 8 and 12 hours, fixed, hydrated, mounted onslides, dipped in photo emulsion, kept under dark conditions for 4 days, developed, fixed and stained. The number of grains per nucleus were counted. Vehicle (acetone) and non-vehicle controls were run. In a second technique, the effect of carbaryl on photolysis-induced DNA fragmentation at 313 nm was measured using alkaline sucrose gradients..

Carbaryl, at concentrations of 1 - 1000 µM, activated UDS both in the absence and presence of S9 microsomes. This was evident at the $p<0.05$ level of significance. For example, after 8 hr, 100 µM carbaryl produced 17.4±2.0 grains / nucleus compared to 2.0±0.7 grains / nucleus in controls. The BUdR experiment produced 0.5, 0.6 and 1.5 breaks/10^8 daltons at carbaryl concentrations of 1, 10 and 100 µM carbaryl, respectively.

This study was considered to be supplemental.

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Onfelt and Klasterska (1984) examined sister chromatid exchanges (SCE) and mutations to thioguanine resistance (TGR) in V79 Chinese hamster cells after treatment with carbaryl. Exponentially growing cells were exposed to 50 or 100 µM carbaryl dissolved in acetone (final [acetone] = 0.25%) for 3 hours. Cultures destined for cytologic examination (SCE) were then treated with BrdUrd at 37°C for 24 hr in the dark, with colcemid added 2 hours before harvest. They were then processed for chromosome visualization. Cultures destined for TGR testing were trypsinized, resuspended and tested with the selective agent.

TGR testing was negative at both carbaryl concentrations. Addition of S9 microsomes or 10 mM glutathione did not change this result, though cellular toxicity was decreased. Carbaryl treatment resulted in a dose-dependent increase in SCE; at ascending doses, the average number of SCEs in cells with 21/22 chromosomes was 7.85±0.27, 10.00±0.47 and 15.06±0.44* (*$p<0.01$). The presence of S9 microsomes attenuated this response.

This study was considered to be supplemental.

5. Formation and genotoxicity / carcinogenicity of nitrosocarbaryl

Eisenbrand *et al.* (1975) have examined the pH dependence of nitrosocarbaryl production from carbaryl and nititrite *in vitro*. 10^{-3} or 10^{-4} M carbaryl was incubated with a fivefold molar excess of nitrite at 37°C in a closed vessel. The target pH values were maintained by addition of HCl or NaOH in a pH-stat, with the reaction stopped after 15 or 60 minutes by addition of amidosulfonic acid. After extraction with dichloromethane and roto-evaporation of the organic phase, the *N*-nitrosocarbaryl was quantitated colorimetrically against a prepared standard.

The carcinogenicity of *N*-nitrosocarbaryl was also examined in this study. Eight male and 8 female Wistar rats received single subcutaneous injections of 1000 mg/kg *N*-nitrosocarbaryl, with 8 controls of unspecified sex treated with the Livio oil vehicle. *N*-nitrosocarbaryl was also administered by gavage to 37 male and female rats (22 rats of either sex served as untreated controls) at single doses ranging from 200 to 1500 mg/kg in 10% starch paste.

At 10^{-3} M carbaryl / 0.1 N HCl / pH 1, the conversion to *N*-nitrosocarbaryl was 1.2% and 1.7% at 15 and 60 minutes, respectively. At pH 2 that conversion was 0.1 and 0.2%, while at pH 3 it was 0.05 and 0.1%. In the presence of a stronger acid (1 N HCl - the pH was not specified), the conversion was 3.2% and 2.2%, with the decline at 60 minutes attributed to instability under those conditions (maximum *N*-nitrosocarbaryl stability occurs at pH 3-5). At 10^{-4} M carbaryl / 0.1 N HCl / pH 1, the conversion was 0.9% at 60 min.

Fifteen of the 16 animals treated subcutaneously with 1000 mg/kg *N*-nitrosocarbaryl developed tumors at the injection site, with 14 of those animals dying by day 450. One animal was still tumor-free at 630 days. In contrast, the single oral administration produced no toxic effects and no tumors through 21 months.

This study is considered supplemental.

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Regan *et al.* (1976) investigated the ability of carbaryl and nitrosocarbaryl to induce DNA single-strand breaks in cultured human skin fibroblasts. The fibroblast DNA was labeled by overnight incubation with ^{3}H-thymidine or with $^{32}PO_2$. The labeled cells were incubated with 10^{-4} M carbaryl or 10^{-4} M NO-carbaryl for 1 hour followed by two washes in growth medium and assayed either immediately or after 20 hours of further incubation. 10,000 cells were layered onto an alkaline sucrose gradient in 0.2 ml of 1 M NaOH, allowed to stand for 1 hour and centrifuged for 3 hr at 30,000 rpm. Extracted DNA was also subjected to cesium chloride

gradient centrifugation for 65 hours at 30,000 rpm.

Sucrose gradient sedimentation profiles of cellular DNA, both immediately after and 20 hours after a 1-hour exposure to 10^{-4} M carbaryl, showed no differences from untreated cells. Similar treatment with NO-carbaryl, however, showed a marked decrease in sedimentation rate both immediately after and 20 hours after exposure. This was interpreted as evidence for induction of single-strand breaks or induction of alkali-labile bonds. A similar result was obtained after a 2-minute NO-carbaryl incubation. Further analysis generated a conclusion that the alkali breaks occurred every 8-18x10^6 daltons. Cesium chloride analysis using ^{3}H- and ^{14}C-labeled NO-carbaryl suggested that the methyl residue, as opposed to the naphthalene ring residue, bound irreversibly to the DNA.

This study was considered to be supplemental.

Table III-10. Genotoxic effects of carbaryl

Test type / system	Species / strain / culture	Dose or concentration	S9	Result	Comments / Reference
Gene mutation:					
Ames test, *S. typhimurium* (*in vitro*)	TA 1535, 1537, 1538, 98, 100	Trial 1: 0, 5, 10, 50, 100, 500, 1000 µg/plate; Trial 2: 0, 10, 50, 100, 500, 1000, 2000 µg/plate	±	Negative	*Acceptable* [a] Lawlor (1989)
Ames test, *S. typhimurium* (*in vitro*)	TA 98, 102, 1535	0, 1, 5, 10, 50, 100, 500, 1000, 5000, 10,000 µg/plate	±	Negative	*Supplemental* [a] Grover *et al*. (1989)
CHO/HGPRT forward mutation assay (*in vitro*)	Chinese hamster ovary cells	2 trials, 0 - 0.3 mg/ml	±	Negative	*Acceptable* Young (1989)
Ouabain resistance (*in vitro*)	V79 Chinese hamster fibroblasts	0.1 - 1000 µM	-	**Positive**	*Supplemental* Ahmed *et al*. (1977a)
Thioguanine resistance (*in vitro*)	V79 Chinese hamster fibroblasts	0, 50 or 100 µM	±	Negative	*Supplemental* Onfelt & Klasterska (1984)
Chromosomal aberration:					
Dominant lethal mutations (*in vivo*)	Rat	#1: in feed at 0, 7, 25,100 or 200 mg/kg/day #2: by gavage in corn oil at 0, 3, 7 25 or 100 mg/kg/day #3: in feed containing corn oil at 0 or 100 mg/kg/day	n/a	Negative	*Supplemental* Weil (1972)
Aberration test (*in vitro*)	CHO-WBL cells	-S9: 0, 7.5, 10, 25, 50 or 75 µg/ml +S9: 0, 150, 200, 250 or 300 µg/ml	±	-S9: Negative +S9: **Positive**	*Acceptable* Murli (1989)
Aberration test (*in vitro*)	Chinese hamster fibroblasts	3 doses, including the 50% growth inhibition dose (max. effective dose = 0.03 mg/ml)	-	**Positive**	*Supplemental* Ishidate & Odashima (1977)
Micronuclei in bone marrow RBCs (*in vivo*)	CD-1 mice	0, 50, 100 or 200 mg/kg/day (for two consecutive days)	n/a	Negative	*Acceptable* Marshall (1996)
Aberration test (*in vivo*)	Root meristems of *Allium cepa*	0, 0.1, 0.4, 0.7, 1.0 or 1.3%	± [b]	**Positive**	*Supplemental* Grover *et al*. (1989)
Mitotic spindle abnormalities (*in vitro)*	V79 Chinese hamster fibroblasts	0, 25, 50, 100, 200 or 400 µM	-	**Positive**	*Supplemental* Soderpalm-Berndes & Onfelt (1988)

DNA damage:					
Unscheduled DNA synthesis (*in vitro*)	SV-40 transformed human cells (VA-4)	0, 1, 10, 100 or 1000 µM	±	**Positive ± S9**	*Supplemental* Ahmed *et al*. (1977b)
Unscheduled DNA synthesis (*in vitro*)	Primary hepatocytes from Fischer 344 rats	Trial 1: 0, 0.5, 1, 2.5, 5, 10, 25 µg/ml Trial 2: 0, 5, 7.5, 10, 15, 20 or 25 µg/ml	-	Negative	*Acceptable* Cifone (1989)
Sister chromatid exchange (*in vitro*)	V79 Chinese hamster fibroblasts	0, 50 or 100 µM	±	**Positive** (particularly w/o S9)	*Supplemental* Onfelt & Klasterska (1984)
Protein and DNA binding in liver (*in vivo*)	CD-1 mice (♂)	^{14}C-carbaryl @ 75 mg/kg either as a single dose or after 13 days of 8000 ppm dietary carbaryl	n/a	Negative	*Acceptable* Sagelsdorff (1994)

[a] The designation "Acceptable" indicates that the study was successfully completed according to FIFRA guidelines. "Supplemental" indicates that the study was not conducted according to FIFRA guidelines; however, such studies were reviewed and considered to contribute to the general genotoxic picture of the chemical.

[b] S14 microsomes from wheat seedlings were used in this study**.**

F. REPRODUCTIVE TOXICITY

1. Overview

Laboratory animal studies from the older Russian literature (1965-1974) revealed histopathologic changes in male reproductive tissues and sperm in various species after exposure to carbaryl. Unfortunately, with two exceptions (the studies of Rybakova [1966] and Shtenberg and Rybakova [1968], which are summarized below), these studies were not available in useful translation. Nonetheless, recent studies by Pant *et al.* (1995, 1996) using rats exposed by gavage, were confirmatory. Several US-based studies have been negative for male reproductive and histopathologic effects, though they employed a dietary, as opposed to a gavage, exposure regimen. This raised the possibility that the method of oral exposure is a crucial determinant for carbaryl toxicity in the male reproductive system. In addition, many of the latter studies did not examine sperm morphology, making it impossible to determine the histopathologic status of the reproductive system.

In light of these mixed results from laboratory animal studies, an early epidemiologic investigation of testicular function among carbaryl-exposed factory workers was undertaken (Wyrobek *et al.*,1981). This study was designed to determine if spermatogenic effects have occurred in human occupational settings. The results of Wyrobek's study suggested that carbaryl may induce spermatogenic toxicity. Furthermore, a more recent study of carbaryl factory workers from China showed statistically higher levels of sperm chromosomal aberrations and DNA damage in an occupationally exposed population (Xia *et al.,* 2005). In two studies, Meeker *et al.* (2004a and 2004b) noted an association between 1-naphthol levels in the urine and certain sperm toxicity parameters (decreased sperm concentrations, decreased sperm motility and increased DNA single strand breaks resulting in high Tail% in comet assays). However, it was not known for certain if the 1-naphthol originated as carbaryl or as napthalene. Possible reproductive effects of carbaryl were also considered in a recent epidemiologic study of male pesticide exposure and pregnancy outcome among farm families in Ontario, Canada (Savitz *et al.,* 1997). In that study, the adjusted odds ratio for miscarriage rose in conjunction with carbaryl exposure, suggesting that exposure of reproductive-aged males could result in clinically manifested toxicity.

All of these studies are summarized below and in Table III-10.

2. Human epidemiologic studies

Wyrobek *et al.* (1981) examined semen samples for spermatogenic abnormalities from a cohort of 50 male carbaryl factory workers (current employees or former workers with at least one year of factory experience) and 34 controls (workers providing samples as part of their pre-employment medical examinations). The men were assigned to one of three exposure groups: control (*i.e.*, the new hires), low dose (supervisors, foremen, substitutes and maintenance workers) and high dose (full-time baggers and operators), though many of the comparisons were made only between controls and exposed workers (*i.e.*, low dose and high dose combined). Rankings were also done according to (1) the number of years of work with carbaryl and (2) whether or not the exposures were current or had occurred in the past (*i.e.*, "previously exposed workers"). The latter group represented 19 of the 49 total exposed men analyzed for sperm morphology; these workers exhibited an average time since employment of 6.3±3.9 years (range = 1-12 years).

The carbaryl air concentrations in the facility were not determined for this study, though air sampling data generated by the company's industrial hygiene program provided an indication of the range of values encountered. Thus three samples from the operations area yielded air concentrations between 0.36 and 14.21 mg/m^3 (mean = 4.9 mg/m^3), 22 samples from the

distribution area ranged between 0.03 and 1.8 mg/m^3 (mean = 0.347 mg/m^3), and 36 personal monitoring samples from the same area ranged between 0.0 and 1.8 mg/m^3 (mean = 0.439 mg/m^3).

A single semen sample was collected per participant after three days of sexual abstinence. Sperm counts and ejaculate volumes were determined. Sperm morphologic defects were elucidated histologically on 500 fixed and stained sperm / sample. Fluorescence assays were conducted to determine the number of sperm carrying double fluorescent bodies, considered evidence for the presence of two Y chromosomes (an abnormality likely due to meiotic nondisjunction). Blood samples were collected to determine testosterone, FSH and LH levels. The roles of possible confounding factors such as age, smoking, recent illness and drug intake were elucidated by multiple regression analysis. Correlations among semen parameters, blood parameters and personal histories were identified using correlation analysis.

Statistically significant differences between groups in sperm counts were not observed. For example, the mean count for entire control group was 128.7x10^6/ml (n=34), compared to 140.7x10^6/ml (n=48) for the exposed group. Age-matched groups (18-40 yr) showed counts of 124.7x10^6/ml (controls, n=33) *vs.* 120.3x10^6/ml (exposed, n=26). However, a non-statistically significant elevation of oligospermic individuals (*i.e.*, those with sperm count < 20x10^6/ml) was observed in the exposed group (control = 2/34 *vs.* exposed = 7/48; p=0.1) which may have biological significance.

There was an increase in the number of abnormally shaped sperm in the carbaryl-exposed population. For example, control samples for the entire 18-40 yr group showed 41.8±2.2% of sperm rated as abnormal (n=33), while the parallel value for the 18-40 yr currently exposed samples was 57.9±3.4% (n=18; p<0.001). Similarly, control samples from the 18-40 yr group *without* confounding factors showed 42.0±2.7% of sperm rated as abnormal (n=22), while the parallel value from the 18-40 yr currently exposed samples *without* confounders was 56.2±4.3 (n=14; p<0.01). The percentage of abnormal sperm in currently exposed men grouped in the high exposure group (n=19) was not appreciably different from currently exposed men grouped with the low exposure group (n=11), though both groups differed from controls (n=34).

Among the 30 currently exposed workers examined, there was a significant negative correlation between the number of years exposed to carbaryl and the percent abnormal sperm (r= -0.42, p<0.025). The authors provided three possible explanations for this unexpected finding: (1) longer-term workers had graduated to less-exposed positions, (2) biologic or pharmacologic adaptation had occurred with the longer exposures (*eg.*, repair processes had been induced), and (3) over time, selection for less-affected males had occurred. None of these rationales were explored in the study.

The authors also recognized a statistically significant negative correlation between age and percent abnormal sperm in the currently exposed group (r= -0.55, p<0.005). As the mean age of the carbaryl-exposed group (40.7±10.0 yr) statistically exceeded that of the controls (26.6±5.6 yr, p<0.001), the negative correlation between age and percent abnormalities was strong evidence that the higher age of the exposed group did *not* account for the increased percent abnormalities in that group. They note, in addition, that a statistically significant correlation between age and percent abnormal sperm was not seen among the controls (r=0.07).

Previously exposed workers, defined above as men with an average time since carbaryl-related employment of 6.3±3.9 years (range = 1-12 years), showed a somewhat higher incidence of abnormal sperm than controls, though this achieved statistical significance only when the entire cohort was considered (*i.e.*, when confounders were included). The proportion of teratospermic men, defined as those with greater than 60% abnormal sperm forms, rose in the exposed population from 4/34 in controls to 9/30 in currently exposed and 5/19 in previously

exposed men. When the two latter groups were combined, creating a teratospermic incidence of 14/49, statistical significance was not achieved (p=0.06).

Assays for fluorescent bodies were conducted on semen samples from 17 high exposure men and 17 controls. While, as expected, these groups showed statistically different percent abnormal sperm percentages (41.2±2.5% in controls *vs.* 52.6±3.6% in high exposure group; p<0.01), there was no statistically significant difference in percent sperm with two fluorescent bodies (0.8±0.2% in controls *vs.* 1.0±0.3% in exposed) nor in percent sperm with one fluorescent body (44.7±0.9% in controls *vs.* 44.3±1.0% in exposed).

Attempts to correlate sperm abnormalities, sperm with double fluorescent bodies, FSH, LH and testosterone, failed. However, a correlation was seen between exposed men with sperm counts of less than $80x10^6$ sperm/ml and percent abnormal sperm. There were 18 men in the sub-$80x10^6$/ml category, showing 64.0±3.8% abnormal sperm *vs.* 29 men in the plus-$80x10^6$ sperm/ml category, who showed 43.6±1.8% abnormal sperm, p<0.01. While this correlation did not track carbaryl exposure history, it did suggest a relationship between shape abnormalities and sperm counts.

These data reveal a correlation between the percent abnormal sperm and exposure to carbaryl under occupational circumstances. It is unclear if the extent of this effect would lead to reproductive or teratogenic problems in individuals, though the authors cite other studies in human populations that correlate spontaneous abortions, reduced sperm counts and marked increases in sperm abnormalities. The strength of this study was limited by small group sizes, imperfect knowledge of the actual exposure concentrations and the possibility that unknown xenobiotics played a role in the exposed cohort. Nonetheless, these results are considered to reflect a carbaryl-mediated effect, especially as several possible confounders were pursued and eliminated.

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Savitz *et al.* (1997) examined pregnancy outcomes in Ontario farm families as a function of farm activities or pesticide exposures that occurred to the adult husbands within 3 months of conception. This time window was appropriate for capturing effects mediated indirectly through damage to sperm. Pregnant mothers older than 44 years were not included. Using the 1986 Canadian Census of Agriculture, 2946 couples from 2693 eligible farms were identified, with 3984 pregnancies ultimately examined.

Exposure was classified using a self-administered activities checklist which included reference to the use of specific pesticides (including carbaryl) by the husbands. A judgement was made concerning the plausibility of direct pesticide exposure exceeding one month. A positive judgement led to a classification as “exposed”. Four possible pregnancy outcomes were enumerated: miscarriage, small for gestational age (SGA), preterm delivery and sex ratio. Odds ratios were calculated using the group of men with “no activity” or “no chemical activity” as the referent populations.

Carbaryl usage, when combined with activities defined generally as “crop herbicide application”, produced an adjusted odds ratio of 1.9 for miscarriage (95% confidence limits, 1.1-3.1). If carbaryl usage was combined with the reporting category of “application of crop insecticides and fungicides”, the adjusted odds ratio rose to 2.1 (95% confidence limits, 1.1-4.1). “Application of crop insecticides and fungicides” alone (*i.e.*, without carbaryl usage) resulted in an adjusted odds ratio of 1.1 (0.8-1.6). Combination of carbaryl usage with use of “yard herbicides” produced an adjusted odds ratio of 1.3 (95% confidence limits, 0.6-2.5). None of the other pregnancy outcome parameters was associated with an elevated odds ratio.

While the odds ratios were consistent with a role for carbaryl-induced sperm damage in miscarriage, the exposure conditions were not well understood and the reported odds ratio

ranges too great to establish clear effects. Consequently, the usefulness of this study in a risk assessment context was limited to providing support for other data that provide a clearer link to genotoxic or male reproductive effects.

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Meeker *et al.* (2004a) studied the relationship between urinary levels of 1-naphthol (1N, a primary carbaryl metabolite as well as a metabolite of napththalene) and various sperm parameters in humans. Subjects were recruited from a pool of 330 men seeking diagnoses from a Boston infertility clinic. They were primarily white (82%), 36.2±5.5 years, 72% never having smoked. Subjects were excluded if they had highly concentrated or dilute urine samples as determined by creatinine concentrations or specific gravity. The chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol (TCPY) was also measured, though those results will not be discussed here.

The parameters measured included sperm concentration, motility and morphology. The morphologic parameters were generated using 200 sperm / donor under the “Tygerberg Strict Criteria”. The motion parameters were measured by computer-aided semen analysis (CASA), incorporating the following outcomes: VAP (mathematically smoothed velocity) and VSL (straight line velocity) - both measures of sperm “progression” - and VCL (curvilinear velocity), ALH (amplitude of lateral head displacement) and BCF (beat cross frequency), measures of sperm “vigor”. Several of the motion parameters were combined to portray “straightness” (STR = [VSL÷VAP] x 100) and “linearity” (LIN = [VSL ÷ VCL] x 100).

Odds ratios (OR) for spermatotoxicity increased when comparing dichotomized sperm parameters from men in the lowest 1N urinary tertile with those in the middle and high tertiles. For example, ORs for below-reference sperm concentrations were $1.0^{\#}$, 4.2* and 4.2* at increasing 1N urinary tertiles (*$p<0.05$; $^{\#}p<0.01$ for trend). ORs for sperm motility were $1.0^{\#}$, 2.5* and 2.4* (*$p<0.05$; $^{\#}p<0.01$ for trend). No statistically significant effect was seen on sperm morphology, though there was a suggestion of an effect: 1.0, 1.4 and 1.6.

Of the CASA motion parameters examined, a statistically significant inverse association was identified for urinary 1N and VSL (regression coefficient = -1.64; $p<0.02$). Inverse associations also existed for VCL (-1.98) and LIN (-0.79), though statistical significance was not attained.

The strengths of the study resided in its size, high participation rate and the use of biologically relevant markers of exposure. Weaknesses included the inability to unambiguously identify carbaryl as the source of 1N and the use of a single urinary sample to estimate the 3-month carbaryl (or naphthalene) exposure. However, with respect to the latter, the authors cite their own *in press* study showing that this was indeed a valid indicator of exposure. In addition, it was difficult to know if the subject population, which consisted of men seeking diagnoses for their infertility, biased the results. In any event, it appears that an interquartile increase in urinary 1N level may be associated with a 4% decrease in sperm motility. This could generate an increase in subfertile men among those whose sperm are already trending toward the bottom of the motility spectrum.

In a parallel study, Meeker *et al.* (2004b) tracked DNA damage in human sperm as a function of 1-naphthol and TCPY concentrations in the urine (TCPY is a metabolite of chlorpyrifos; as in the previous study, the TCPY results will not be summarized here). DNA damage was assessed using a modified "comet" assay, which is based on the electrophoretic distances traveled by negatively-charged DNA molecules from single sperm cells toward a positive electrode. These movements form a characteristic comet tail, the shape of which is indicative of toxic damage due to the creation of DNA fragments, etc. Two hundred and sixty subjects were recruited from

a pool of 368 men seeking diagnoses in a Boston infertility clinic. They were primarily white (82%), with a mean age of 36.1±5.6 years. 74% of the cohort had never smoked, while 9% were current smokers. Subjects were excluded if they had concentrated or dilute urine samples. This was determined primarily by urinary specific gravity measurements, but also by urinary creatinine concentrations. Of the original subject pool, there were 19 azoospermic men (*i.e.*, semen with no sperm cells) among a total of 74 subjects whose semen was, for various reasons, not analyzed. 1N was determined in a single urinary sample from each subject.

The comet assays were performed under neutral conditions with 50 µl semen-agarose mixtures embedded between additional layers of agarose on electrophoretic glass slides. After dissolving the cell membranes in lysing solution and treating with RNase and proteinase K to dissolve chromatin, the slides were subjected to electrophoresis for 1 hr, fixed, dried, stained and observed under a fluorescence microscope. Comet tail parameters, including comet extent, tail distributed moment (TDM; an integrated measure of the distance and intensity of comet fragments) and percent of the total DNA in the tail (Tail%), were established for 100 sperm / sample using specialized computer software. Cells with tails greater than 300 µm were too long to analyze with the software. As this condition (CHD) was considered to result from severe DNA damage, such cells were enumerated and used as an additional measure of DNA damage.

A highly statistically significant association was found between Tail% and 1N: the regression coefficient was 4.13 (p=0.0003; 95% confidence limits, 1.92-6.32). Thus for an interquartile range increase in 1N, the Tail% significantly increased by 4.13%. Regression coefficients were negative for comet extent and TDM, but they did not achieve statistical significance. However, stratifying the data by comet extent revealed a statistically significant negative association between Tail% and TDM. This suggested that there was at least some association between 1N and TDM. However, the apparent inverse relationship was unexpected. Since TDM is an integrated value (dependent both on distance and intensity), the authors speculated that it may reflect the *type* of DNA damage that occurred. For example, a cell that has high Tail% and low TDM may reflect a predominance of single strand breaks, while a cell with low Tail% and high TDM may reflect a predominance of double strand breaks. The authors' analysis suggests that carbaryl produces single strand breaks resulting in high Tail%.

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Xia *et al.* (2005) examined the question of whether carbaryl exposure to workers in a pesticide factory in Changzhou, China disposed them to spermatotoxicity. The study included a total of 46 sperm donors, age 21-48 years, all nonsmokers and nonregular drinkers. Sixteen were carbaryl workers who had both worked in the plant for more than 1 year and had worked there continuously for the 6 months prior to sampling. An internal control group of 12 individuals worked in the same complex, but were isolated from the pesticide facility. An external control group of 18 individuals with no history of carbaryl exposure came from other professions. There were no significant age or work year differences between groups.

The following sperm parameters were gauged: semen volume, sperm concentration, sperm number, sperm motility (using the CASA program referred to above in Meeker *et al.*, 2004a) and sperm morphologic abnormalities (using fixed and stained sperm). Modified TUNEL assays (deoxy-nucleotidyl transferase-mediated dUTP-biotin nick end-labeling) were performed to determine the percent DNA fragmentation. Multicolor FISH assays (fluorescence *in situ* hybridization) were performed to detect chromosome aberrations on the X and Y chromosomes and on chromosome 18 using DNA probes specific to the centromeric regions.

No significant differences were noted for semen volume, sperm concentration, sperm number and sperm motility. Morphologic abnormalities did exhibit a statistical increase, with 20.50±6.71% among internal controls, 15.92±7.58% among external controls and

25.25±4.90%** among the carbaryl-exposed group (**p<0.01 compared to external controls). This was due primarily to statistically significant increases in head abnormalities (9.85±5.12% vs. 7.56±3.61% vs. 11.72±4.93%*; p<0.05) and tail abnormalities (8.73±4.10% vs. 6.60±4.78% vs. 10.82±3.09%**).

TUNEL assays showed a statistically significant increase in the percentage of cells with fragmented DNA in the carbaryl-exposed population: 13.36±12.17% in internal controls, 13.92±7.15% in external controls, 21.04±8.88%* in the carbaryl-exposed group (p<0.05 compared to both internal and external controls).

Disomic sperm appeared to increase in the carbaryl-exposed group as revealed in the FISH assays. Thus the percentage of XY18 sperm in internal control, external control and carbaryl-exposed groups was 0.280±0.076%, 0.177±0.080% and 0.281±0.102%** (p<0.01 compared to external controls). The percentage of YY18 sperm was 0.134±0.052%, 0.079±0.042% and 0.185±0.083%** (p<0.01 compared to external controls; p<0.05 compared to internal controls). The percentage of X1818 sperm was 0.093±0.053%, 0.051±0.028% and 0.113±0.070%* (p<0.05 compared to external controls). The percentage of Y1818 was 0.076±0.031%, 0.052±0.043% and 0.119±0.055%** (p<0.01 compared to external controls; p<0.05 compared to internal controls).

The FISH assays also revealed a possible increase in nullisomic sperm in the carbaryl-exposed group. The percentage of sperm nullisomic for sex chromosomes in internal control, external control and carbaryl-exposed groups was 0.383±0.099%, 0.277±0.077% and 0.426±0.174%** (p<0.01 compared to external controls). The percentage of sperm nullisomic for chromosome 18 was 0.222±0.062%, 0.139±0.043% and 0.268±0.126%* (p<0.05 compared to internal controls).

These results were consistent with a role for carbaryl in the induction of chromosomal aberrations and DNA fragmentation in human sperm. Carbaryl also appeared to be associated with an increased incidence of men with sperm morphologic abnormalities.

3. Laboratory animal studies

a. Contract laboratory studies

A two-generation reproductive toxicity study in CD rats was conducted by Tyl *et al.* (2001). F_0 animals, 30/sex/dose (enough to yield at least 20 pregnant rats/dose), received carbaryl (99.1% purity) in the feed for 10 weeks at doses of 0, 75, 300 and 1500 ppm *ad libitum*. Calculated carbaryl intakes for the F_0 and F_1 parental animals were 5-6 mg/kg/day at 75 ppm, 21-36 mg/kg/day at 300 ppm and 92-136 mg/kg/day at 1500 ppm. The pre-breeding period was followed by a 2-week mating period within treatment groups, with exposure continuing, to produce the F_1 generation. F_0 males were necropsied at the time of delivery. F_1 litters were culled to 10 on postnatal day (pnd) 4 and weaned on pnd 21, at which time the F_0 females were necropsied along with up to 3 F_1 weanlings/sex/litter. Thirty F_1 parental animals/sex/dose were then chosen to produce the F_2 generation. They were exposed to dietary carbaryl at the same doses for 10 weeks, mated within their dose groups over a 2-week period, and the F_1 parents and F_2 pups sacrificed and analyzed over the same time spans as for the previous generation. Standard observations were made at appropriate intervals for clinical signs, body weight changes, feed consumption, reproductive performance, reproductive system histology / histopathology and organ weights.

There were no treatment-related clinical signs in either sex. Precoital and gestational periods were comparable among groups of both generations. Mean estrus cycle length was slightly longer in F_0 females at 1500 ppm (4.77 days) compared with controls (4.59 days), but (1) the effect was not statistically significant, and (2) estrus cycle lengths among F_1 females

were similar across dose groups. In light of the human epidemiological studies, the older Russian studies, and those of Pant *et al.* (1995, 1996), all summarized in this section, it is pertinent to mention that there were no effects on F_0 or F_1 parental epididymal sperm counts, motility, morphology, homogenization-resistant spermatid head counts, daily sperm production or efficiency of daily sperm production.

Food consumption among F_0 males was slightly decreased at 1500 ppm, with some intervals showing statistical significance when expressed on a g/day basis. However, consumption on a g/kg/day basis was similar across dose groups. F_0 females showed no differences in food consumption. Similar to the F_0 males, F_1 males showed lower g/day food consumption at 1500 ppm, though g/kg/day consumption was actually higher. The same was true for F_1 females.

Mean pre-breeding body weights were consistently statistically suppressed at 1500 ppm among F_0 and F_1 animals of both sexes. These were largely reflective of statistical decrements in weight gain during the first pre-breeding week of exposure (Table III-11). There was also some indication that weight gains among the 300 ppm animals were suppressed, particularly among F_1 males. Maternal body weight gains were suppressed at 1500 ppm in both the F_0 and F_1 generations, though lactational weight gains appeared little affected, or were increased, by carbaryl exposure. Mean F_1 and F_2 pup body weights tended to be suppressed at 1500 ppm, particularly as the lactational period progressed (Table III-11, pup body weights, postnatal days 0 and 21).

Pup survival measurements indicated possible treatment effects at 300 and 1500 ppm. Though the data were not as statistically robust as the body weight data, statistical linear trends were detected in survival indices for F_1-day 14 pups, F_2-day 4 pups and F_2-day 7 pups (Table III-11). The mean number of live pups / litter, F_1-pnd 4 (precull) did not appear affected. However, the same parameter for the F_2 pups was statistically depressed at the mid and high doses (Table III-11).

Both male and female F_1 pups appeared to sustain statistically significant developmental delays at 1500 ppm. In males this was represented by a delay in the time of preputial separation, while in females it was represented by a delay in vaginal opening (Table III-11). Because the F_2 pups were sacrificed at weaning, similar measurements were not made for them. However, anogenital distance measurements on F_2 pups did not suggest an effect.

Necropsies on parental animals and pups, F_0- F_1-F_2, did not reveal treatment-related effects.

The parental NOEL was set at 75 ppm (5-6 mg/kg/day), based on reduced body weight gains at 300 and 1500 ppm. Because no effects on reproductive indices were detected, the reproductive NOEL was set at >1500 ppm (92-136 mg/kg/day). The pup NOEL was set at 75 ppm, based on increased pup mortality, reduced body weights and delayed developmental indices at 1500 ppm and increased F_2 mortality, pnd 0-4, at 300 and 1500 ppm. This corresponded to a parental intake of 5-6 mg/kg/day, though nothing is known of the carbaryl intake in the pups (carbaryl concentrations in milk were not determined).

This study was acceptable by FIFRA guidelines.

Table III-11 . Effect of dietary carbaryl on reproductive parameters in CD rats (Tyl *et al.*, 2001)

	Carbaryl concentration in diet (ppm) [a]			
	0	**75**	**300**	**1500**

Body wt. gain, pre-breeding days 0-7 (g)				
F_0 ♂	58.9	58.6	54.6	45.2**
F_0 ♀	24.5	24.5	23.4	17.9***
F_1 ♂	64.6	63.1	58.6*	54.2**
F_1 ♀	40.1	40.4	37.8	36.3*
Body wt. gain, gestation days 0-20 (g)				
F_0 ♀	138.1	130.2	132.0	121.0***
F_1 ♀	140.1	137.2	134.3	115.3***
Body wt. gain, postnatal days 0-21 (g)				
F_0 ♀	9.3	3.3	12.6	14.4
F_1 ♀	8.5	5.7	10.8	15.8
Pup body wts. (g)				
F_1, postnatal day 0	6.34	6.68	6.41	6.09
F_1, postnatal day 21	48.79	49.46	50.73	43.46***
F_2, postnatal day 0	6.27	6.51	6.58	6.00
F_2, postnatal day 21	50.91	52.30	49.68	40.39***
Pup survival index (%)				
F_1, 4-day	98.4	99.1	95.0	98.1
F_1, 7-day	99.7	100.0	99.6	99.3
F_1, 14-day	99.7^{+}	100.0	99.2	95.4
F_2, 4-day	98.3^{++}	98.7	92.0	88.9
F_2, 7-day	100.0^{+}	99.6	96.0	93.0
F_2, 14-day	98.6	99.6	94.8	96.6
Mean live pups / litter (precull)				
F_1, postnatal day 4	13.9	13.3	13.9	14.3
F_2, postnatal day 4	15.4^{++}	13.9	12.7*	12.5**
Pup developmental indicators				
F_1 ♂, day of preputial separation [b]	41.6	41.5	41.7	43.7**
F_1 ♀, day of vaginal opening [b]	30.6	31.3	31.3	32.0**

*, **, ***: p<0.05, 0.01, 0.001

$^{+}$, $^{++}$: p<0.05, 0.01 (trend test)

[a] Calculated carbaryl intakes for the F0 and F1 parental animals were 5-6 mg/kg/day at 75 ppm, 21-36 mg/kg/day at 300 ppm and 92-136 mg/kg/day at 1500 ppm.

[b] Because the F_2 pups were sacrificed at weaning, similar developmental measurements were not made for them.

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b. Studies from the open literature - oral gavage exposure

Rybakova (1966) studied the effects of gavage dosing with carbaryl (100% purity) in rats ("mixed albino") and mice (acute only; strain not stated). Control animals were treated with the vehicle, sunflower oil. Acute, subchronic and chronic exposure regimens were employed. As many of the observed effects involved reproductive tissues, this study is most relevant to this section of the RCD.

Acute. The LD_{50} for female rats and mice was 437.5±70.6 mg/kg and 515±79.2 mg/kg, respectively. Clinical signs were not reported.

Subchronic. Male and female rats (32/sex/dose) were treated by gavage with 0 and 50 mg/kg/day carbaryl for 50 days. The following effects were noted in exposed animals: (1) 8-

10% weight gain decrement, (2) decrements in butyrylcholinesterase (27%) and acetylcholinesterase (41%; the tissue of origin was not stated), (3) significant decrease in adrenal ascorbic acid levels (amount not stated; $p<0.001$), (4) 40.4% mean decrease in spermatozoa motility, (5) 11.3% mean delay in the estrus cycle, (6) 50% mean prolongation of diestrus, (7) a decrease in the "period of heat" from 1.34 to 1.05 days, (8) increase in the excretion of gonadotropic hypophyseal hormones was reported in immature mice, though neither the causative dose nor the specific hormones was reported, (9) adrenal and liver weights were increased by 20% and 28%, respectively.

Chronic. In the chronic phase of this study, 24 rats/sex/dose were treated by gavage with carbaryl at 0, 7, 14 and 70 mg/kg/day for 12 months. Toxicity testing was performed at 3, 6, 9 and 12 months. The following observations were made: (1) no overt toxicity, (2) 6-8% decrement in weight gain at the low and mid doses, with the high dose generating a "statistically significant loss of weight ($p<0.001$) throughout the experiment" (data not provided), (3) mean cholinesterase activities decreased by 3.3%, 32.5% and 94% at 12 months (tissue of origin not stated), (4) 15-30% increase in adrenal weight at the low and mid doses and a 43% rise at the high dose, paralleled by a dose-dependent expansion of the zona glomerulosa, with unusual mitotic patterning, (5) adrenal ascorbic acid levels lowered by non-statistically significant amounts, (6) sperm motility inhibited in a dose-dependent fashion, achieving statistical significance at the mid and high doses at 6 and 9 months, and at all three doses at 12 months (% inhibition of motility at 6 months at increasing doses: 5%, 13%**, 40%***; 9 months: 7%, 16%**, 56%***; 12 months: 22%***, 36%***, 74%***; **,***$p<0.01$, 0.001), (7) seminiferous tubules with a dose-dependent edema of the interstitial tissue, desquamation of the spermatogenic epithelium and destruction of the parenchyma, (8) estrus cycle lengths increased by 10%, 20% and 70%, resulting from increases in the diestrus phase length, (9) increased number of corpora lutea and atretic follicles (data not provided), (10) a bioassay suggested that carbaryl exposure increased the secretion of gonadotrophic hormones (few details provided), (11) other histopathologic effects noted in liver and kidneys.

While many effects were indicated by this study, very little actual data were provided. Nonetheless, the evidence for reproductive toxicity was supportive of similar findings from other studies (particularly oral gavage studies) and thus considered relevant in the current context.

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Shtenberg and Rybakova (1968) administered carbaryl (100% active material) by oral gavage to albino rats (strain not identified) on a daily basis for 12 months. Doses were 0 (vehicle and volume not identified), 7, 14 or 70 mg/kg/day. Initial examinations were followed by observations conducted at 3-month intervals. The following parameters were evaluated: survival, general health, motor activity, body weight, blood butyryl- and acetylcholinesterase, duration of spermatozoal motility, status of the estrus cycle (vaginal smears), gonadotropic function, terminal adrenal, thyroid and reproductive organ histology, adrenal lipid content, thyroid function, pituitary (hypophyseal) glucoprotein analysis, and stress testing (by fasting) at termination.

Statistically significant body weight gain decrements were detected "throughout the experiment" at 14 and 70 mg/kg/day ($p<0.001$), but not at 7 mg/kg/day (data not provided). Blood butyryl- and acetylcholinesterase were inhibited from the first measurement at 3 months.

The "duration of spermatozoal motility" was statistically suppressed at the two high doses at 6 and 9 months, and at all three doses at 12 months (duration of motility at increasing doses, 12 month assay: 40.6, 31.5***, 25.8***, 10.6*** min). Histopathologic changes, observed in the testes at all doses in a dose-dependent fashion, included edema of interstitial tissue, destruction and desquamation of germinal epithelium and reduction in the number of

spermatocytes and spermatids.

Gonadotropic function was evaluated in a bioassay involving the injection of homogenized pituitary glands from treated animals into immature recipient mice. According to the report (p. 464), “whereas the hypophyseal homogenate from rats given carbaryl at a level of 7 mg/kg/day for 12 months increased the weight of the ovaries and uterus in immature recipient mice by an average of 23 and 49%, respectively, the hypophyseal homogenate from rats given 70 mg carbaryl/kg increase the weight of the ovaries by 51.5% and of the uterus by 123% compared with the weight of these organs in control mice.” The report goes on to claim that histology of hypophyses from treated rats showed evidence of increased cell size, loss of granules and hyalinization of the cytoplasm. The authors feel that these changes were at the root of the observed reproductive gland disturbances.

Histopathologic changes in the adrenal glands of rats receiving 7 mg/kg/day were also reported (p. 464): “an increase in the size and mitotic activity of cells in the zona glomerulosa. Enlarged cells, either binuclear or containing a large nucleus, were present in the fascicular zone. There was also an increase in lipids compared with the controls.”

By 3 months, the length of the estrus cycles in high dose females was statistically increased. By 6 months, both mid and high dose animals had statistically longer cycles, a situation that was maintained through 12 months (estrus cycle length, 12 months: 4.56, 5.07, 5.81** and 7.75*** days; **, ***$p<0.002$, 0.001).

Absorption and excretion of ^{131}I in the thyroid was also impaired by carbaryl exposure, indicated “by a reduction in the rate of absorption and excretion of ^{131}I and its rather low recovery, in comparison with the controls. Thus at the two lowest dosage levels, ^{131}I-absorption reached a peak within the first 4-6 hr and represented on average 16% of the administered ^{131}I. In contrast, corresponding figures in the control group were 2-4 hr and 18%, while in animals given 70 mg carbaryl/kg/day the peak of ^{131}I-absorption was reached only after 20 hr and represented 10.2% of the administered dose. After 24 hr, rats on the two lowest dose levels had absorbed, on average, 10.5-9.6% of ^{131}I, as against 10.4% in the controls. In contrast, rats on the highest level had absorbed much less iodine (only 6.8%; $p<0.001$). After reaching a peak, thyroid activity began to decrease gradually. The slower rate of ^{131}I-absorption at the 70 mg/kg/day level may be regarded as an indication of a decrease in the functional activity of the thyroid. In the thyroids of these animals, the follicular epithelium in the central areas was flattened, follicles were enlarged and the colloid was more dense and basophilic... In the peripheral areas, changes in the follicular epithelium and colloid were less pronounced. At the 7 and 14 mg/kg/day levels, too, the structure of thyroid tissue differed from that of the controls, although to a lesser degree than in the rats receiving 70 mg/kg/day.” (pp. 464-466).

The authors hypothesize that the endocrine effects noted in this study may have been secondary to effects on the pituitary gland. The (subchronic) LOEL for this study was 7 mg/kg/day, based on reduced sperm motility, effects on hypophyseal and thyroid function, and hypophyseal, adrenal and thyroid histopathology. A NOEL was not set.

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Dikshith *et al.* (1976) treated male albino rats (strain not stated, though they originated in a colony maintained by the Industrial Toxicology Research Centre, Lucknow, India) with carbaryl (99.0% pure) by oral gavage at doses of 0 (1 ml peanut oil) and 200 mg/kg, 3 days/week, for 90 days. There were 7 animals/dose. Histopathologic analysis was conducted on liver, kidney, testes and epididymis. Biochemical analysis was also conducted as follows: liver and testes - succinic dehydrogenase, adenosine triphosphatase, alkaline phosphatase and acid phosphatase; liver - glucose-6-phosphatase; brain and blood - AChE. A further 7 animals/dose were mated with unexposed females after the 90-day exposure period. When the females were

deemed pregnant (by examination of vaginal smears for sperm), they were separated and allowed to complete the pregnancy. Litters were evaluated for weight and numbers of pups born. Pups were observed for 10 days *post partum*.

Though one animal each from the control and treated groups died on days 18 and 32, respectively, there were no signs of carbaryl-induced toxicity in any animal throughout the study. The report further states (p.163) that (1) "there were no gross abnormalities in the liver, kidney, testis, and epididymis of the experimental rats" and (2) "microscopic examination of these organs also did not present significant histological changes". However, one of the micrographs showed a testicular tubule from a treated rat apparently filled with debris along with a more general enlargement of the interstitium (Fig. 4, p. 166). It is not clear from the report that histopathology was actually carried out on control tissues.

The following enzymes showed statistically significant changes when assayed after the 90-day period (*p<0.05; **p<0.01; ***p<0.001): testis - succinic dehydrogenase (control *vs.* treated, 2.96±0.17 *vs.* 3.49±0.13* nm/min/mg protein), adenosine triphosphatase (72.31±1.61 *vs.* 83.43±3.83* nm/min/mg protein); liver - glucose-6-phosphatase (83.08±4.25 *vs.* 100.16±5.41* nm/min/mg protein); blood - AChE (8.15±0.45 *vs.* 5.30±0.56*** µmol/ml/10 min); brain - AChE (0.96±0.02 *vs.* 0.85±0.04* µmol/100 µl of 10% homogenate/10 min).

Though no data were provided, the report states that there were no significant effects on the rate of pregnancy, litter size, number of offspring born, or on pup health and viability through 10 days.

The report minimizes the importance of any of the histological or biochemical changes noted above. It does not explain the apparent pathology noted in the abovementioned testicular micrograph. While it is possible that gavage treatment of male rats did not precipitate overt effects on fertility or pup viability, it is unclear why such a high dose (200 mg/kg; *i.e.*, very near the LD_{50}), provided 3 times per week over a 90-day period, did not result in clinical signs. Since no analytical data were available, one cannot be sure of the actual dose delivered.

This report has clear inadequacies in data reporting and analytical analysis. It is included here because it specifically examined male rat reproductive tissues after gavage treatment with carbaryl.

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Kitagawa *et al.* (1977) treated four male Wistar rats by gavage with carbaryl (purity not stated). The dose was applied for one year at 3 mg/rat/week (it is assumed that this was done with a single weekly dosing, though the actual dosing regimen was not stated in the report). With an approximate weight of 200 g at the start of the study, this would have been equivalent to about 15 mg/kg/week, or about 2 mg/kg/day if administered daily (though the report did not provide this information). Four control rats were gavaged with physiological saline (volume not provided). The pancreas, adrenal gland and testis were analyzed for histopathologic changes following sacrifice.

Examination of the testicular slides indicated "an obvious reduction in the number of the cells in the seminiferous tubules, especially in spermatogonia and in spermatozoa" (p. 55.) A micrograph from a treated and from a control testis seemed to support this statement, though the prevalence of the effect (*i.e.*, the number of animals affected *vs.* controls) was not reported. There also appeared to be a reduction in the number and size of Langerhans islets in the pancreas. Effects on the adrenals were unremarkable.

A summary of this study was included here because of the attempt to detect testicular histopathology, which is relevant to the discussion of potential carbaryl-induced reproductive effects.

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Narotsky and Kavlock (1995) examined the possible reproductive and developmental effects of carbaryl (purity, 99%), along with nine other xenobiotics, in pregnant Fischer 344 rats. Animals were treated by gavage between gestation days 6-19 inclusive. Carbaryl doses were 0 (corn oil vehicle, 21 rats), 78 (16 rats) and 104 (16 rats) mg/kg/day. The high dose was selected based on companion study which provided evidence for toxicity in nonpregnant females. The low dose was set at 75% of the high dose. The animals were observed throughout the study for toxicity. Maternal body weights were determined on gestation days 6, 8, 10, 13, 16 and 20. Pups were examined and counted on post natal days 1, 3 and 6, and collectively weighed on post natal days 1 and 6.

Tremors, motor depression and lacrimation were noted, usually during the first three days of treatment, while jaw clonus (repetitive contractions) occurred throughout the treatment period. As the dose levels corresponding to these signs were not explicitly reported, it is assumed that they occurred at both treatment doses. The first two days of treatment resulted in statistically significant weight losses at both doses (data were only expressed graphically; weight gains at 0, 78 and 104 mg/kg/day were ~2.5, ~-8*** and ~-9*** g, respectively; ***p<0.001), while the gestation day 6-20 period produced a statistically significant decrement at the low dose and a statistically significant loss at the high dose (~16.5 g, ~8 g** and ~-1.5 g***; **,*** p<0.01, 0.001). Pup weights were suppressed by ~6% at the high dose on postnatal day 1 (p<0.001). By pnd day 6 there were no statistically significant differences among dose groups, though the mean high dose litter weights were ~5% less than controls. Two of the 13 pregnant dams (15%) sustained complete resorption at the high dose.

In this study, the observed developmental toxicity of carbaryl occurred at doses that induce parallel maternal toxicity.

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Pant *et al.* (1995) administered carbaryl (99.2% purity) by oral gavage to male Wistar rats on a 5-days/wk basis for 90 days. The doses were 0 (0.2 ml peanut oil), 50 or 100 mg carbaryl/kg/day, 8 rats/dose. After terminal sacrifice on day 91, the reproductive organs were removed and weighed. One testis/rat was preserved for histopathology, while the other was homogenized for assay of testicular enzymes. Sperm counts and motility determinations were carried out using epididymal sperm.

Carbaryl-exposed rats were reportedly lethargic, though no details were provided as to doses, timing or numbers of animals affected. Body weights were reportedly statistically depressed by 60 days at the high dose, showing a >20% deficit by day 90 (data were only presented graphically). No effects on testicular, accessory sex organ or epididymidal weights were observed, though again, actual data were not provided.

Testicular glucose-6-P-dehydrogenase (associated with premeiotic germ cells) and sorbitol dehydrogenase (associated with pachytene spermatocyte maturation) were suppressed at the high dose (G6PDH activities at ascending doses: 89.1, 79.1, 26.3* nmol/min/mg protein; SDH: 3.18, 2.94, 1.63*; p<0.05). Testicular lactate dehydrogenase (associated with germline elements of the testes; inversely proportional with sperm maturation) and γ-glutamyl transpeptidase (marker enzyme for Sertoli cell function) were statistically increased at both doses (LDH: 244, 390*, 500* nmol/min/mg; γGT: 23.2, 37.3*, 58.3*).

Total epididymal sperm counts and percent sperm motilities were statistically decreased at both doses (sperm counts/epididymis: $10x10^7$, $6x10^7$*, $4x10^7$*; sperm motility: 89.5%, 67.5%*, 33.1%*). The total percent sperm abnormalities were increased at both doses (18.7%, 46.3%*, 56.0%*), reflecting increases for each type of abnormality (banana head, detached head, neck curved, curved, bent, tail round, tail short, tail looped).

Carbaryl caused several histopathologic changes in the testes. These included congestion, edema, depressed spermatogenesis and accumulations of cellular and acellular masses in the seminiferous tubular lumen.

The LOEL for subchronic toxicity was <50 mg/kg/day, based on testicular enzyme, sperm and testicular histopathologic changes. This study was considered to be supplemental.

Pant *et al.* (1996) conducted a follow-up study to establish a NOEL for spermatotoxic effects in the rat and to determine if young rats were more susceptible to such effects than older rats. Six young and 6 old male Druckrey rats/dose were exposed by gavage to carbaryl (99.2% purity) at 0 (0.2 ml peanut oil), 25, 50 or 100 mg/kg/day, 5 days/week, for 60 days. Body weights were determined at initiation and at terminal sacrifice (day 61), after which the reproductive organs (testes, epididymides, seminal vesicles, ventral prostate and coagulating glands) were removed and weighed.

The authors state that no overt toxicity was detected and that weight gains were suppressed at 50 and 100 mg/kg/day, though the actual data were not supplied. The young rats exhibited statistically significant absolute weight deficits at 100 mg/kg/day for the testes, epididymides, seminal vesicle, ventral prostate and coagulating gland, though again, the data were not provided. This was apparently not the case for the adult rats. Relative weight deficits were not observed in either the adult or the young rats.

Effects on sperm parameters were seen only at 50 and 100 mg/kg/day, and may have been severe in the young rats, though the data on this aspect were not robust. Sperm counts per epididymis in young rats were, at ascending doses, $8.0x10^7$, $8.2x10^7$, $6.0x10^7$* and $5.0x10^7$* ($p<0.05$). In older rats they were $8.0x10^7$, $8.5x10^7$, $7.0x10^7$*and $6.0x10^7$*. Percent motile sperm in young rats was 86.0%, 85.0%, 65.0%* and 49.1%*, while in older rats it was 88.3%, 85.8%, 75.0%* and 65.0*. Percent abnormal sperm in young rats was 10.5%, 11.3%, 19.8% and 33.7%, while in older rats it was 10.3%, 11.1%, 16.1% and 23.1% (apparently statistical significance was not achieved). According to the report, some abnormalities (bent up or down acrosomes) appeared only in the younger rats.

The NOEL for damage to the male reproductive system was set at 25 mg/kg/day, based on a LOEL of 50 mg/kg/day. This study was considered supplemental.

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c. Studies from the open literature - dietary or intraperitoneal exposure

Collins *et al.* (1971) studied the reproductive effects of carbaryl in Mongolian gerbils (*Meriones unguiculatus*), comparing the results to a parallel study in Osborne-Mendel rats (data not summarized here). Gerbils were fed diets containing carbaryl (99% pure) at 0, 2000, 4000, 6000 or 10,000 ppm for 100 days starting at weaning. Forty control pairs were then mated, along with 30 pairs for each dose group except for the high dose, which had 18. Non-survival of high dose F_{2b} males made it necessary to reuse F_{2a} males to generate the F_{3b} generation. Litters were observed on the day of birth to determine the number of stillborn and liveborn young and for abnormalities. They were observed again on *post partum* day (ppd) 4 for number and condition of the living pups. At weaning, F_{3a} and F_{3b} animals from the 0 and 6000 ppm groups were preserved for histopathology.

Impairment of fertility was evident at the high dose, becoming certain with the F_2 generation. Statistically significant effects at other doses were less clearly related to exposure [9].

[9] Data for the parameters discussed in the summary of the Collins *et al*. study in gerbils are as follows:

The mean number of pups per litter was convincingly decreased at the high dose, though statistically significant decrements were also noted at 2000, 4000 and 6000 ppm. The mean number of liveborn pups per litter exhibited similar behavior, *i.e.*, significant, but not clearly dose-responsive, effects at dose levels as low as 2000 ppm and clear effects at 10,000 ppm. The mean number of survivors to day 4 was reduced at all dose levels. The mean number of survivors to day 21 was probably also reduced at doses as low as 2000 ppm. Weanling weights were decreased at 4000 ppm and up. This was particularly true for males.

Adult body weights and food consumption were not monitored in this study, precluding calculation of internal doses. Nonetheless, using 100 g as the average adult gerbil weight and 8 g/day as the average food consumption (Harkness and Wagner, 1983), the high dose of 10,000 ppm would correspond to an internal dose of approximately 800 mg/kg/day, with the lower doses proportionally smaller. Though clear dose responsiveness was not evident in some cases, a reproductive LOEL was set at 2000 ppm (~160 mg/kg/day) based on statistically significant decreases in the mean numbers of liveborn pups per litter, the mean number of survivors to days 4 and 21, and the mean weanling weights at that dose and above. However, the internal dose calculations assume that these relatively high dietary carbaryl levels had no effect on food consumption or body weight. As this assumption could not be proven, the resultant internal doses, as well as the calculated internal dose LOEL, should be viewed with caution and only in support of more authoritative data. While the authors were persuaded that parallel rat data indicated that rats may have been less sensitive than gerbils, this was not altogether clear from inspection of that data. At any rate, in view of the very high doses and the unusual rat strain, the rat data were not summarized for this document.

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Fertility index at increasing doses, 1st mating-2nd mating, F_1 generation: 95%-89%, 83%-84%, 77%*-78%, 93%-79%, 83%-64%; F_2 generation: 98%-100%, 97%-93%, 93%-93%, 90%-93%, 60%*-33%**; F_3 generation: 100%-98%, 87%*-81%*, 93%-89%, 95%-81%*, 50%*-0%**; *p<0.05, **p<0.01.

Mean litter size, 1st mating-2nd mating, F_1 generation: 4.90-4.26, 4.27-3.60, 3.97-4.13, 4.50-3.61, 4.00-3.00; F_2 generation: 5.70-5.23, 5.07-4.55, 4.47*-4.79, 4.50*-5.00, 3.20*-1.33*; F_3 generation: 5.68-5.27, 4.43**-4.12**, 4.93-4.61, 5.14-4.21, 3.00-0.00*; *p<0.05, **p<0.01.

Mean liveborn per female mated, 1st mating-2nd mating, F_1 generation: 4.67-4.13, 4.13-3.56, 3.67-4.00, 3.80-3.36, 3.06*-2.57; F_2 generation: 5.60-5.10, 4.80-4.24, 3.90**-4.50, 4.03**-4.56, 3.00**-1.33**; F_3 generation: 5.60-4.27, 4.20**-4.00*, 4.60*-4.39, 4.59-3.86**, 3.00-0.00**; *p<0.05, **p<0.01.

Mean survivors to day 4 per female mated, 1st mating-2nd mating, F_1 generation: 3.55-3.32, 2.37-2.68, 2.20*-2.57, 2.23*-1.93*, 0.94**-1.00**; F_2 generation: 4.70-4364, 3.57*-3.31*, 2.30**-2.46**, 1.97**-2.30**, 1.40**-0.67**; F_3 generation: 4.52-4.47, 3.03**-3.12*, 2.33**-2.50**, 2.82**-1.81**, 1.50*-0.00**; *p<0.05, **p<0.01.

Mean survivors to day 21 per female mated, 1st mating-2nd mating, F_1 generation: 3.30-3.16, 2.33-2.48, 2.07-2.43, 2.10-1.86, 0.56**-0.79**; F_2 generation: 4.17-4.26, 3.43-3.17, 2.17**-2.18**, 1.60**-2.15**, 1.20**-0.67**; F_3 generation: 4.02-3.95, 2.73**-2.77*, 2.07**-2.36**, 2.27**-1.76**, 1.50-0.00**; *p<0.05, **p<0.01.

Mean male weanling weight in grams, 1st mating-2nd mating, F_1 generation: 15.1-14.5, 15.4-15.6, 11.1*-14.0, 13.1*-13.6, 13.2**-13.4; F_2 generation: 14.1-13.9, 13.6-13.3, 13.6-14.0, 13.0-13.3, 11.8**-NSW [no survivors to weaning]; F_3 generation: 14.2-14.4, 13.4-14.4, 12.9**-13.4, 13.3-12.9, 11.5**-NSW; *p<0.05, **p<0.01.

Jordan *et al*. (1975) subjected male Balb mice to daily injections of Karbatox 75 (carbaryl; purity not stated) for 10 and 20 days (propoxur was also tested, though those data will not be discussed here). The number of treated mice was not reported. The dose was 20 mg/kg/day.

No histopathologic effects were seen in testicular, liver or kidney sections, nor were there karyotypic changes in spleen or testicular tissues. However, there were statistically significant increases in nuclear volume in neurosecretory cells of the hypothalamus (nucleus supraopticus: 362.57 μ^3 in controls, 408.61 μ^3 in treated animals; nucleus paraventricularis: 312.18 μ^3 in controls, 359.34 μ^3 in treated animals) and in the number of Gomori stained-positive glial cells per 0.076 mm^2 of the nucleus habenulae (controls: 5.75, treated animals: 6.88). The authors speculated that the increase in Gomori stained cells was a response designed to protect the brain from xenobiotic-induced toxicity. No speculation was offered to explain the cell volume changes, but it might be inferred that they may lead to further endocrine or reproductive effects.

This summary was included because it attempted to measure potential testicular histopathology, which is relevant to the discussion of possible carbaryl effects, and because it provided evidence for possible effects on neurosecretory cells in the brain.

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Osterloh *et al.* (1983) examined the testicular effects in C57BL mice of intraperitoneal exposure to 10 separate pesticides, including carbaryl (99.8% purity). Four of these compounds were known testicular toxins (dibromochloropropane, dinitrobutylphenol, Ordram and Benomyl) and three were known mutagens (dibromochloropropane, chlorbenzilate and atrazine). Carbaryl was administered on 5 consecutive days to 4 male mice/dose at 0 (corn oil alone),12, 25, 50, 100, 200, 400 and 800 mg/kg/day. The mice were sacrificed on day 35, and the morphology of 200 sperm per mouse assessed by oil immersion microscopy. In addition, testicular weights and total epididymal sperm counts were determined. Methyl methanesulfonate served as the positive control substance.

Carbaryl had no effect on any of the testicular parameters measured, even at levels above the LD_{50} for this compound (LD_{50} = 108-650 mg/kg; Cranmer [1986]). Similarly, none of the other compounds elicited testicular toxicity. The authors speculated that this assay may be relatively insensitive to the effects of recognized testicular toxins, either because the mouse was resistant to testicular effects of these particular xenobiotics or the assay was improperly timed *vis a vis* the spermatogenic cycle. Though the authors don't mention it, the intraperitoneal route may also not be optimal for testicular toxicity. In any case, the apparent negativity of intraperitoneally administered carbaryl on sperm morphologic parameters in this study should be viewed with caution, particularly in the context of positive observations from other studies.

Table III-12. NOEL and LOEL values in laboratory animal studies on the reproductive toxicity of carbaryl

Species, strain	Study type & exposure regimen	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
CD rat	2-gen. repro., dietary	parental: ⇓ wt. gains repro.: no effect at HDT [a] pup: ⇑ F_2 mortality	parental: 5-6 mg/kg/day repro.: >92-136 mg/kg/day pup: 75 ppm [b]	parental: 21-36 mg/kg/day repro.: >92-136 mg/kg/day pup: 300 ppm [b]	*Acceptable* Tyl *et al.* (2001)
Rat (strain not identified)	12-month oral gavage	⇓ sperm motility, effects on hypophyseal & thyroid function, and adrenal & thyroid histopath.	<7 mg/kg/day (LDT) [a]	7 mg/kg/day (LDT) [a]	*Supplemental* Shtenberg & Rybakova (1968)
Wistar rat (♂ only)	90-day oral gavage	changes in testicular enzyme levels, and sperm / testicular histopath.	<50 mg/kg/day (LDT)[1]	50 mg/kg/day (LDT)[1]	*Supplemental* Pant *et al.* (1995)
Druckrey rat (♂ only)	60-day oral gavage	damage to the ♂ reproductive system	25 mg/kg/day	50 mg/kg/day	*Supplemental* Pant *et al.* (1996)
Mongolian gerbil	100-day dietary	repro.: ⇓ # liveborn pups, ⇓ # survivors (days 4-21), ⇓ weanling weights	repro.: <2000 ppm (~160 mg/kg/day) (LDT) [a, c]	repro.: 2000 ppm (~160 mg/kg/day) (LDT) [1,3]	*Supplemental* Collins *et al.* (1971)

[a] HDT, high dose tested; LDT, low dose tested

[b] This value is expressed as a dietary concentration because it was not possible to determine actual carbaryl intake in the pups.

[c] The internal dose levels in the gerbil study were calculated using published values for average body weight and food consumption in that species (Harkness and Wagner, 1983). Use of these default body weight and food consumption values was based on the unproven assumption that they were unaffected by the carbaryl intake.

G. DEVELOPMENTAL TOXICITY

1. Overview

Developmental toxicity studies in rats, rabbits and mice did not indicate specific problems stemming from carbaryl exposures. There were, however, indications from two studies in the open literature that carbaryl is a developmental toxin in beagle dogs. The extent to which developmental toxicity occurs in the absence of maternal toxicity and the relevance of the dog in the context of a human health risk assessment are considered in later sections of this assessment.

The results of the developmental toxicity studies are summarized in Table III-13.

2. Laboratory animal studies

a. Rats - gavage

Pregnant, sperm positive, CD rats, 25/dose group, received carbaryl (99.0% purity) by gavage at 0 (0.5% methylcellulose 400; 10 ml/kg), 1, 4 or 30 mg/kg/day, on gestation days (gd) 6-20 (Repetto-Larsay [1998]). Maternal body weights were determined on gd 0 and 6-21. Clinical observations were performed daily. The dams were sacrificed on gd 21, after which gravid uterine weights, number of corpora lutea and number and status of implantations were determined. Live fetuses were removed and examined and their placental weights measured. Approximately half of the live fetuses were fixed and dissected for internal examination. The remaining half were eviscerated, fixed and stained for skeletal examination.

No deaths occurred in any dose group. At 30 mg/kg/day, 18/25 dams registered at least one occurrence of increased salivation within 20 minutes of administration, disappearing by about 1 hr. This observation was made primarily between gd 14 and 20, though in two animals it was noted as early as gd 7. Statistically significant decrements in maternal body weight gain were noted at 30 mg/kg/day over the entire gestation period (weight gains at ascending doses, gd 6-21: 132.76, 137.76, 132.48, 96.88** g; **p<0.01), with marked effects noted within one day of the commencement of dosing (weight gains, gd 6-7: 3.16, 4.76, 3.04, -3.12** g; **p<0.01). These effects were accompanied, and probably caused by, decreases in food consumption at the high dose (food consumption at ascending doses, gd 6-9: 27.74, 28.04, 28.49, 22.90** g/day; **p<0.01). Clear treatment effects were not evident for the following parameters: maternal necropsies, corpora lutea, implantations, preimplantation loss, post implantation loss, resorptions, dead fetuses and gender ratio. Mean fetal body weights were reduced in a statistically significant manner at the high dose for both males and females (mean male fetus weights: 5.56, 5.56, 5.46, 5.10** g; mean female fetus weights: 5.24, 5.20, 5.25, 4.87** g). The number of live fetuses classified as runts (defined as those with body weight $\leq$75% of control means) rose at the high dose, with a statistically significant effect in evidence when the data were expressed on a per litter basis (incidence of runts, fetal data: 0/377, 3/389, 3/377, 8/389; litter data: 0/25, 2/25, 3/25, 6/25*; *p<0.05). Incomplete or absent ossification of the 7th cervical centrum, incomplete ossification of the 5th sternebra and non ossification of the 1st metacarpae were increased at 30 mg/kg/day. These were considered to reflect the lower fetal weights at the high dose, which in turn may have resulted from lower maternal weight gains at that dose.

The maternal NOEL was set at 4 mg/kg/day, based on clinical signs and suppressed body weight gains at 30 mg/kg/day. The developmental NOEL was also 4 mg/kg/day, based on lower fetal body weights and ossification delays at 30 mg/kg/day. This study was acceptable by FIFRA guideline standards.

b. Rabbits and mice - gavage

Timed-pregnant New Zealand White rabbits were exposed to carbaryl (99% purity) by gavage on gestation days (gd) 6-29 (Tyl *et al.* [1999]). There were 22 animals/dose. Doses were 0 (0.5% aqueous methylcellulose; 2 ml/kg), 5, 50 or 150 mg/kg/day. Dose selection was based on a rangefinding study using 100 mg/kg/day as the high dose. In that study, plasma ChE was inhibited to 41% of control and RBC ChE to 80.1% of control (not statistically significant) at 10 mg/kg/day.

Clinical observations were made twice daily during the dosing period. Maternal body weights were determined every three days between gd 0 and 27, and again on gd 29 and 30. Food consumption w as monitored throughout. Blood was drawn for plasma and RBC ChE determinations on gd 25. Terminal sacrifices were carried out on gd 30, 1-1.5 days before parturition, followed by maternal necropsy and determination of litter and fetal status.

There were no deaths attributed to carbaryl exposure, though one doe each at 0 and 5 mg/kg/day and two does at 50 mg/kg/day died on days 29 and 30. There were also no maternal clinical signs attributed to exposure. Maternal weight gains were statistically significantly suppressed at 150 mg/kg/day for gd 6-9 (at ascending doses: 69.4, 62.4, 31.3, -74.8** g; **p<0.01), 29-30 and 6-30 (529.1, 524.4, 453.3, 325.9** g). Weight gains also tended to be less at 50 mg/kg/day, though statistical significance was not achieved. Food consumption was either not notably affected or was, at 50 mg/kg/day, increased (food consumption, gd 6-29: 41.6, 43.6, 49.8**, 40.0 g/kg/day). Both plasma and RBC ChE, measured on gd 25, were suppressed at 50 and 150 mg/kg/day by statistically significant margins (plasma ChE: 211, 183, 114**, 67** mU/ml; RBC ChE: 1083, 1019, 679**, 796** mU/ml).

Uterine examinations revealed no treatment effects on numbers of corpora lutea, implantation sites, pre- or postimplantation loss, number of live fetuses/litter or gender ratio. However, the mean fetal body weights were statistically suppressed at the high dose (♂: 52.69, 51.29, 50.48, 47.44* g; ♀: 50.40, 49.76, 50.42, 45.34** g). While the number of fetuses with skeletal malformations showed a small increase at the high dose (0/153, 0/174, 0/137, 2/171), both observations were due to fused sternebrae and occurred in a single litter. This could not be clearly ascribed to carbaryl exposure.

The maternal NOEL was set at 5 mg/kg/day, based on suppression of RBC and plasma ChEs at 50 and 150 mg/kg/day. The developmental NOEL was set at 50 mg/kg/day, based on reduced fetal body weights observed at 150 mg/kg/day. This study was considered to be acceptable by FIFRA guidelines.

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Murray *et al.* (1979) examined the teratogenic potential of carbaryl (99.0% purity) in New Zealand White rabbits and CF-1 mice after oral gavage (both species) or dietary (mice only) exposure. Doses were designed to approximate MTDs determined in preliminary studies. Pregnant rabbits were treated by gavage with 0 (1 ml cottonseed oil/kg), 150 or 200 mg/kg/day carbaryl on gestation days (gd) 6-18. Pregnant mice were either treated by gavage with 0 (5 ml cottonseed oil/kg), 100 or 150 mg/kg/day on gd 6-15, or through the diet to 0 or 2830 ppm on gd 4-5 and to 0 or 5660 ppm (~1166 mg/kg/day) on gd 6-15. There were 13-20 rabbits/dose (two separate groups were run as concurrent controls with each dose) and 23-44 mice/dose (includes both exposure routes). The animals were observed daily during gestation for clinical signs, with maternal body weights established at predetermined intervals. Conventional observations were conducted for pregnancy status and fetal condition (external, soft tissue and skeletal observations).

Rabbits, gavage. Diarrhea was observed at 200 mg/kg/day only (quantitative data not provided). One death occurred among the controls and one among the 150 mg/kg/day group. These were not attributed to carbaryl exposure. Both controls and dosed animals lost weight

over the gestation day 6-11 period, though the latter decrements were statistically greater than the controls (controls *vs.* 150 mg/kg/day, gestation days 6-11: -0.03±0.09 kg *vs.* -0.15±0.10* kg; controls *vs.* 200 mg/kg/day, -0.03±0.07 kg *vs.* -0.31±0.10* kg; *p<0.05).

There was no effect on the mean number of live fetuses per litter, though there was a marginal, non-statistically significant increase in resorptions at both doses (resorptions per litter, control *vs.* 150 mg/kg/day, 0.8±1.2 *vs.* 1.3±2.8; control *vs.* 200 mg/kg/day, 0.5±1.1 *vs.* 1.5±1.9).

Fetal body weights were reduced at both doses, though only the lower dose achieved statistical significance (control *vs.* 150 mg/kg/day, 37.9±5.4 g *vs.* 34.0±3.4* g; control *vs.* 200 mg/kg/day, 39.2±4.2 g *vs.* 36.7±3.8 g). Fetuses also were slightly smaller in size, though not by statistically significant margins (fetal crown-rump length, control *vs.* 150 mg/kg/day, 93.5±5.9 mm *vs.* 91.1±3.6 mm; control *vs.* 200 mg/kg/day, 96.3±4.1 mm *vs.* 93.8±3.6 mm). Omphalocele[10] occurred at statistically higher rates among the 200 mg/kg/day animals than among controls (control *vs.* 150 mg/kg/day, total fetuses [total litters], 0/113 [0/14] *vs.* 1/149 [1/17]; control *vs.* 200 mg/kg/day, 0/113 [0/13] *vs.* 6/82 [4/12]*). The four dams that gave birth to pups exhibiting this malformation sustained the greatest gestation day 6-11 mean weight loss (440 g). Single cases of omphalocele, hemivertebrae and conjoined nostrils with missing nasal septum were observed at 150 mg/kg/day, but statistical significance was not achieved.

The developmental LOEL in rabbits was 150 mg/kg/day based on the increase in omphalocele at 150 and 200 mg/kg/day. The single incidence of this malformation at 150 mg/kg/day was considered to be exposure-related because of the extremely low incidence among historical controls (laboratory historical controls revealed only 2 cases among 338 litters). The maternal LOEL in rabbits was 150 mg/kg/day based on statistically significant weight gain deficits at 150 and 200 mg/kg/day. Neither developmental nor maternal NOELs were determined. As noted in the report (p. 87), “the individual dams which had offspring with omphalocele were among those which demonstrated the greatest degree of maternal toxicity” (though the individual data required to verify this statement were not provided in the report). A similar statement was not made with respect to the single incidence at 150 mg/kg/day. It could not therefore be stated with assurance that omphalocele occurred only in the presence of maternal toxicity.

Mice, gavage and diet. Maternal toxicity was noted at the 150 mg/kg/day gavage dose. There were 10/37* deaths, compared to 0/41 among controls and 1/23 among animals gavaged at 100 mg/kg/day (*p<0.05). In addition, salivation, ataxia and lethargy were noted at 150 mg/kg/day. No clinical signs were noted among controls or low dose animals. Animals exposed to 5660 ppm carbaryl in the feed (~1166 mg/kg/day) exhibited neither deaths nor clinical signs. Mean dam weight gains, gd 6-9, were statistically reduced in the high dose gavage animals (weight gains at 0, 100 and 150 mg/kg/day: 2±1, 2±2 and 0±2* g). Animals exposed through the diet did not show a significant weight gain decrement between gd 6-9 (weight gains at 0 and 5660 ppm: 2±1 and 1±2 g), but did between gd 10-15 (11±4 and 7±4* g). There was a statistically significant increase at the high gavage dose in the number of pregnancies detected by sodium sulfide stain only, a procedure that was conducted only on those animals that appeared not to be pregnant (0/12, 1/2 and 4/7*). Implantations per dam, live fetuses per litter, resorptions per litter and sex ratio were not affected under any treatment regimen. However, fetal body weights were significantly reduced in the group exposed through the diet to 5660 ppm

[10] Omphalocele is defined as a “protrusion, at birth, of part of the intestine through a large defect in the abdominal wall at the umbilicus, the proturding bowel being covered only by a thin transparent membrane composed of amnion and peritoneum” (Dorland’s Illustrated Medical Dictionary, 26th edition, page 921). It is considered to be an external malformation.

carbaryl (1.02±0.12 *vs.* 0.80±0.14* g), as was the fetal crown-rump length (24.1±1.3 mm *vs.* 22.2±1.8* g). Skull and sternebral ossification delays were also reported at that dose, though quantitative data were not provided. The fetal growth and ossification effects in the dietarily exposed animals probably reflected the reduced maternal weight gains during the gd 10-15 period. The dams exposed by gavage did not exhibit such effects, though it is noted that the gavage doses were much lower than the dietary dose. No statistically significant increase in malformations was noted by either exposure route, though two incidences of hemivertebra and fused ribs were noted in the dietarily exposed group.

The developmental NOEL in mice treated by oral gavage was >150 mg/kg/day (no developmental adverse effects were noted). The maternal NOEL in gavaged mice was 100 mg/kg/day based on deaths, cholinergic signs and weight gain deficits (gd 6-9) at 150 mg/kg/day.

A developmental NOEL was not established for mice treated through the diet. The developmental LOEL for dietarily exposed mice was 5660 ppm (1166 mg/kg/day), based on decreased fetal body weights, decreased fetal crown-rump lengths and ossification delays. A maternal NOEL was also not established for these animals. The maternal LOEL was 5660 ppm (1166 mg/kg/day), based on decreased maternal body weight gains.

c. Dogs - dietary

Smalley *et al.* (1968) (with additional discussion and data in Cranmer [1986]) exposed beagle dogs to dietary carbaryl (99.9% pure) at 0, 3.125, 6.25, 12.5, 25 or 50 mg/kg/day. Each dog was fed once daily with 35 g of feed per kg body weight. Females were mated in estrus with one male on day 1 and a second male on day 3. Dosing began between days 3 and 6 after mating, continuing until the end of gestation (avg. gestation length, 62 days). The number of females per dose group varied between 16 (concurrent controls) and 8 (high dose). Clinical observations were made on a daily basis. Body weights were recorded weekly for dams and pups. Necropsies were performed at weaning (8 wk). Cholinesterase activities were not measured.

There were neither clinical signs nor discernable effects on maternal body weights during gestation. Dystocia, defined in this study as a “pattern” of difficult births (delayed delivery accompanied by restlessness, anorexia, fever, and the presence of a green-black, foul-smelling vaginal discharge; also, placental separation and atonic uterine musculature were evident in some cases), was seen in dosed animals, though a dose-response was not apparent: The number of dams showing dystocia / number bred was, at increasing doses: 0/16, 3/10, 3/10, 5/18, 3/9, 3/8. One female from each of three dose groups (6.25, 25 and 50 mg/kg/day) showed evidence of conception but all of the resultant fetuses died. According to the report (p. 396), “The uteri in these cases showed four to six evenly spaced round prominences of the same size in each animal. On incision, it was found that the masses were encapsulated, closely adherent to the uterine mucosa, and composed of yellow-green caseous material with foci of calcification.” The number of implantations per litter and the number of resorptions per litter were reported only for the dosed animals (*i.e.*, not for the controls). Implantations per litter were, at increasing doses: nr (not reported), 8.7, 9.6, 6.1, 6.5 and 6.0. Resorptions per litter were: nr, 3.1, 4.7, 1.2, 2.7 and 2.5. It appeared, therefore, that carbaryl exposure at 12.5 mg/kg/day and above may have caused decreased implantation, though without control data it was not possible to state this with assurance. Conception was notably reduced at the high dose only: 81%, 70%, 80%, 89%, 78%, 37%. No pups were born alive at the high dose: 81%, 66%, 62%, 38%, 60%, 0%.

While the mean pup weights were similar among dose groups at birth, the rate of pup weight gain in the combined dose groups was less than the control group. For example, inspection of the pup weight graph indicates about a 33% disparity between controls and

combined dose groups by week 8 (weaning). Unfortunately, since the mean pup weights at each dose were not provided, it was impossible to determine the minimum dose required for such weight gain effects. All pups exhibited normal avid nursing behavior, though dosed pups cried more and sustained higher mortality.

The percent of pups weaned decreased with dose (73%, 60%, 50%, 23%, 39%, 0%), though the cause of death was not determined. The number of litters containing pups with abnormalities appeared to rise with treatment above 3.125 mg/kg (0/13, 0/7, 1/7, 3/16, 3/6, 1/2; historical controls: 3/313), though the small numbers of animals, particularly at the high dose, precluded a definitive statement of dose responsiveness. The abnormalities included "abdominal-thoracic fissures with varying degrees of intestinal agenesis and displacement, varying degrees of brachygnathia, ecaudate pups [*i.e.*, without a tail], failure of skeletal formation, failure of liver development, and superfluous phalanges" (p. 392).

Thus serious developmental and teratogenic effects of carbaryl were evident in this study. The absence of maternal clinical signs distinct from the dystocia noted at parturition was notable. Limitations on numbers of animals, common for a dog study, curtailed the ability to document dose responsiveness and statistical significance.

The maternal LOEL was set at the low dose of 3.125 mg/kg/day based on the dystocia noted at all dose levels. Consequently, a maternal NOEL was not set, despite the fact that no maternal clinical signs outside of dystocia were observed. Implantations were suppressed at and above 12.5 mg/kg/day, an observation which may indicate either general maternal toxicity or a more specific degeneration of the uterine environment making it unfavorable to implantation. It should be noted that, unlike the FIFRA-compliant rat or rabbit studies, where fetal exposure commences after implantation and is limited to the period of organogenesis, dosing in the present study commenced on gestation day 3 and continued throughout gestation and weaning.

The developmental NOEL was 3.125 mg/kg/day based on teratogenic abnormalities in pups detected at both the litter and individual animal levels at doses as low as 6.25 mg/kg/day. There was insufficient data on pup weight gain decrements to include that parameter as a NOEL determinant. This study is considered to be supplemental.

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Immings *et al.* (1969) studied the effects of dietary carbaryl (purity, 99.84%) in pregnant beagles and their offspring. Four untreated males acted as sires. Dosing commenced on gestation day 1, continuing through pup weaning at 6 weeks of age. Twelve females/group were dosed at 0, 2, 5 and 12.5 mg/kg/day. Body weights were determined weekly. Each animal was presented with 200 grams of dosed feed mixed with 45 grams of canned beef. Food consumption was not recorded and the report does not state whether or not the whole presentation was consumed every day; consequently, there was uncertainty about the actual delivered doses. Gestation length, numbers of viable and stillborn pups and mean litter weights were determined at birth, followed by culling of the litters to six. Mean pup weight was determined at weaning. Pup autopsies were performed only when considered necessary by the veterinarian.

Table III-13 summarizes the maternal and pup data. Of the mated females, 9/12, 7/12, 9/12 and 9/12 became pregnant. One female in each carbaryl-exposed group died. The death reported at 2 mg/kg/day was an animal killed *in extremis* on day 48 due to poor health and convulsions. The mid and high dose deaths occurred at parturition; signs were not reported for those animals. The pattern of pregnancies and maternal deaths did not clearly implicate carbaryl. While the presence of convulsions in the low dose death might be construed as cholinergic, the absence of this sign at higher doses may indicate that it was not related to carbaryl exposure. The time of occurrence (day 48) for a sign that is more likely to be acute in

nature, in addition to the absence of other cholinergic signs, also supported a non-carbaryl-dependent etiology. Effects of carbaryl on maternal body weight were not apparent.

Carbaryl exposure may have increased the incidence of stillbirths at the top two doses ($p<0.01$). There was even a hint of a similar effect at the low dose ($p>0.05$). The increased stillbirth incidence was present at both the fetal and litter levels. It should be noted, however, that all the pups from the two animals dying during parturition were stillborn (high dose female #5030 ⇒ 8 pups; mid dose female #5575 ⇒ 4 pups), as were all 5 pups from one mid dose female (#5202) that aborted on day 41. If these were excluded from the data, the incidence of stillborn pups at increasing doses was 1/45, 3/33, 7/37* and 6/49 (*$p<0.05$). The litter incidence was 1/9, 3/7, 4/7 and 4/8. While these adjusted data were weaker, they remained consistent with a carbaryl-mediated effect, even at the low dose.

Also indicated was an increased number of pup deaths commencing 24 hours after birth in treated groups. The report noted that 18 of these deaths occurred in litters arising from matings that occurred during a specific 2-month time period, arguing that the increase may have been due to an infectious agent (evidence for this could not be deciphered from the report). Since a greater proportion of these particular matings occurred in animals destined to be treated with carbaryl (*i.e.*, only one control litter and one low dose litter were among these matings, compared to 4 mid dose and 4 high dose litters), the investigators felt that the increase among treatment groups constituted a “statistical quirk”. The pup mortality ratios between 24 hours and 6 weeks which exclude these affected litters appear to bear this out. However, removing these animals from consideration is regarded as speculative, as it remains possible that the deaths were related to carbaryl exposure regardless of the presence or absence of infection. It should also be noted that a litter effect did not manifest in these data.

Abnormalities were detected in the pups at the mid and high doses. These included umbilical hernia, cleft palate, fat-like mass in the heart, intussuception of the ileum into the colon, extravasation of blood in the myocardium and unilateral microphthalmia. The report states that, with one exception, all of these signs occurred in litters produced from the allegedly problematic 2-month mating period. However, this cannot be viewed as certain. Finally, two of 8 pups from a mid-dose litter showed incomplete ossification of the 13th rib, though no other skeletal abnormalities were noted.

A developmental LOEL of 2 mg/kg/day was set for this study based on the non-statistically significant increase in stillbirths at that dose. Because it was the low dose, a developmental NOEL was not determined. On a per-litter basis, statistical significance was achieved only at the mid dose, though the incidences at the low and high doses were suggestive of an effect. Also increasing at the mid and high doses were number of viable pup deaths commencing 24 hours after birth and the occurrence of visceral abnormalities among the pups. Carbaryl-related maternal effects were not reported in this study. Consequently, the maternal LOEL was >12.5 mg/kg/day. Because fetal effects occurred at lower doses than maternal effects, carbaryl may be a developmental toxin in the dog. Nonetheless, the problems noted in the study should be recognized.

The primary deficiencies in this study included a lack of dose analysis, no necropsies performed on the mothers, mother’s ages not reported, and the high dose may not have been sufficient. This study is considered to be supplemental.

Table III-13. Effect of subchronic exposure to dietary carbaryl on pregnant beagle dogs and their offspring; Immings *et al.* (1969)

Effects	Dose (mg/kg/day) 0	2	5	12.5
Maternal				
Mated females	12	12	12	12
Number pregnant	9	7	9	9
Maternal deaths	0	1[a]	1[b]	1[b]
Offspring				
Total births	45	33	46	57
Mean births / litter	5.0	4.7	5.1	6.3
Live births	44	30	30	43
Live births / litter	4.9	4.3	3.3	4.8
Stillbirths / total pups [# available litters]	1/45 (2%) [1/9] (11%)	3/33 (9%) [3/7] (43%)	16/46** (35%) [6/9]* (67%)	14/57** (25%) [5/9] (56%)
Deaths (0-24 hr) / total pups [# available litters]	1/44 (2%) [1/9] (11%)	0/30 (0%) [0/7] (0%)	0/30 (0%) [0/7[c]] (0%)	1/43 (2%) [1/8[d]] (13%)
Deaths (24-48 hr) / total pups [# available litters]	0/43 (0%) [0/9] (0%)	2/30 (7%) [1/7] (14%)	2/30 (7%) [2/7[3]] (29%)	4/42 (10%) [3/8[4]] (38%)
Deaths (48 hr - 6 wk weaning) / total pups [# available litters]	5/43 (12%) [4/9] (44%)	10/28* (36%) [3/7] (43%)	9/28* (32%) [5/7[3]] (71%)	12/38* (32%) [5/8[4]] (63%)
Deaths (24 hr - 6 wk weaning) / total pups [# available litters]	5/43 (12%) [4/9] (44%)	12/30** (40%) [4/7] (57%)	11/30* (37%) [5/7[3]] (71%)	16/42** (38%) [6/8[4]] (75%)
Removing affected litters:[e] Deaths (24 hr - 6 wk weaning) / total pups [# available litters]	5/39 (13%) [4/8] (50%)	10/25* (40%) [3/6] (50%)	7/15* (47%) [3/3] (100%)	4/18 (22%) [2/4] (50%)
Total pup mortality [# available litters]	7/45 (16%) [6/9] (67%)	15/33** (45%) [7/7] (100%)	27/46** (59%) [9/9] (100%)	31/57** (54%) [9/9] (100%)

[a] This low-dose mother was killed *in extremis* on day 48 with convulsions and in poor general health.
[b] The mid and high-dose maternal deaths occurred at parturition on days 54 and 61, respectively.
[c] Two mid-dose litters experienced total litter loss at birth. Consequently, the number of available litters was reduced from 9 to 7 for all deaths of fetuses that were born alive.
[d] One high-dose litter experienced total litter loss at birth. Consequently, the number of available litters was reduced from 9 to 8 for all deaths of fetuses that were born alive.
[e] Litters born from matings during a certain 2-month span are removed from consideration here due to the authors' suspicion of illness (see text).

Table III-14. NOEL and LOEL values for studies on the developmental toxicity of carbaryl

Species, strain	Study type & exposure regimen	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
CD rat	oral gavage, gestation days 6-20	maternal: clinical signs (salivation) & suppressed body wt. gains dvp.: ⇓ fetal body wts. & ossification delays	maternal: 4 mg/kg/day dvp.: 4 mg/kg/day	maternal: 30 mg/kg/day dvp.: 30 mg/kg/day	*Acceptable* [c] Repetto-Larsay (1998)
NZW rabbit	oral gavage, gestation days 6-29	maternal: ⇓ RBC and plasma ChE dvp.: ⇓ fetal body wts.	maternal: 5 mg/kg/day dvp.: 50 mg/kg/day	maternal: 50 mg/kg/day dvp.: 150 mg/kg/day	*Acceptable* Tyl *et al.* (1999)
NZW rabbit	oral gavage, gestation days 6-18	maternal: ⇓ body wt. gain dvp.: omphalocele	maternal: <150 mg/kg/day (LDT) [a] dvp.: <150 mg/kg/day (LDT)[1]	maternal: 150 mg/kg/day (LDT)[1] dvp.: 150 mg/kg/day (LDT)[1]	*Supplemental* [c] Murray *et al.* (1979)
CF-1 mouse	oral gavage, gestation days 6-15	maternal: deaths, ⇓ body wt. gain, clinical signs dvp.: no adverse effects noted	maternal: 100 mg/kg/day dvp.: >150 mg/kg/day (HDT)[1]	maternal: 150 mg/kg/day (HDT)[1] dvp.: >150 mg/kg/day (HDT)[1]	*Supplemental* Murray *et al.* (1979)
CF-1 mouse	dietary, gestation days 4-15	maternal: ⇓ body wt. gain dvp.: ⇓ fetal body wts., ⇓ fetal crown-rump length, ossification delays	maternal: <1166 mg/kg/day (HDT & LDT)[1] dvp.: <1166 mg/kg/day (HDT & LDT)[1]	maternal: 1166 mg/kg/day (HDT & LDT)[1] dvp.: 1166 mg/kg/day (HDT & LDT)[1]	*Supplemental* Murray *et al.* (1979)
Beagle dog	dietary, gestation day 3 - parturition (~gd 62) [b]	maternal: dystocia dvp.: teratogenic abnormalities (abdominal-thoracic fissures, brachygnathia, ecaudate pups, failure of skeletal formation, failure of liver development, superfluous phalanges	maternal: <3.125 mg/kg/day dvp.: 3.125 mg/kg/day	maternal: 3.125 mg/kg/day dvp.: 6.25 mg/kg/day	*Supplemental* Smalley *et al.* (1968)
Beagle dog	dietary, gestation day 1 - weaning (pup age 6 wk)	maternal: no adverse effects noted dvp.: ⇑ stillbirths	maternal: >12.5 mg/kg/day (HDT) dvp.: <2 mg/kg/day (LDT)	maternal: >12.5 mg/kg/day (HDT) dvp.: 2 mg/kg/day (LDT)	*Supplemental* Immings *et al.* (1969)

[a] HDT, high dose tested; LDT, low dose tested

[b] Dietary exposure probably continued through weaning, 8 weeks *post partum*, though the report was not explicit on this point.

[c] The designation "Acceptable" indicates that the study was successfully completed according to FIFRA guidelines. "Supplemental" indicates that the study was not conducted according to FIFRA guidelines; however, such studies were reviewed and considered to contribute to the general genotoxic picture of the chemical.

H. DEVELOPMENTAL NEUROTOXICITY

Robinson and Broxup (1997) exposed 32 pregnant CD rats/dose to carbaryl (99.1% purity) by daily gavage from gestation day (gd) 6 through *post partum* day (ppd) 10 inclusive. Doses were 0 (aqueous 0.5% carboxymethylcellulose / 0.1% Tween 80, 10 ml/kg/day), 0.1, 1 or 10 mg/kg/day. Twenty-six animals from each group were examined for developmental neurotoxicity and 6 were examined for cholinesterase activity (plasma, whole blood and brain). The F_1 generation, consisting of 3 males and 3 females, was weaned at day 21.

F_0 animals were checked twice daily for mortality and toxic signs. Body weights were determined on gd 0, 6, 9, 12, 15, 18 and 20, and again on ppd 0, 4, 7, 11, 13 and 21. Modified functional observational batteries were performed 0.5-2 hr post dose on all days in which body weights were determined, excepting ppd 0. Gross pathology was done on ppd 21-23.

Dams destined for cholinesterase determinations were weighed on ppd 10. Blood samples were obtained predose on gd 6, and 1 hr post dose (*i.e.*, at the time of peak effect) on gd 6, 15 and 20, and on ppd 4 and 10 for blood ChE assays. Brains were removed, weighed and analyzed for ChE on ppd 10.

Pups were weighed on ppd 0, 4, 7, 11, 13, 17 and 21. Litters were culled to 4/sex/litter on ppd 4. Tooth eruption was assessed from ppd 7 and eye opening from ppd 12. 1/sex/litter were subjected to neuropathology or brain weight determinations on ppd 11. Motor activity tests were performed in figure-8 mazes on 1/sex/litter for 1 hr on ppd 13, 17 and 21. Litters were weaned on ppd 21 to provide the F_1 adult generation.

F_1 adults were weighed weekly and examined twice daily for mortality and clinical signs. For females, vaginal opening was assessed from ppd 26 until development of this character. For males, preputial separation was assessed from ppd 34 until development of this character. Motor activity was assessed on ppd 60. Auditory startle habituation was measured on ppd 22 & 60. Passive avoidance tests were conducted on ppd 23 and 24. “E” water maize testing was conducted between ppd 60 and 65. Animals not selected for the F_1 generation were sacrificed, necropsied and brain weights determined at weaning. Brains from 6 high dose and 6 control pups/sex were subjected to histopathology and brain morphometry. At approximately 10 weeks of age, at least 12/sex/group underwent perfusion fixation. Neuropathology was conducted on a given fraction of these animals. Neural morphometry was conducted on a further 6 F_1 adults from the control and high doses.

Results, F_0 animals (only females tested). There were neither treatment-related deaths nor signs detected in twice daily examinations. Reduced weight gains were noted at 10 mg/kg/day for the gd 6-9 period: 6.6, 7.7, 7.2 and 0.5** grams (**p<0.01) at ascending doses. FOB testing at 10 mg/kg/day revealed an increased incidence in dams with pinpoint pupils on all occasions during the dosing period (p<0.05-0.005), as well as in dams with slight tremors or slight ataxic gait on many occasions (Table III-15a). Slight hypotonic gait also increased at 10 mg/kg/day (statistically significant at gd 18, p<0.01), and possibly increased at 1 mg/kg/day (statistically significant at gd 12, p<0.05). However, the overall gait data were not sufficiently robust at 1 mg/kg to make a definitive determination on this point. A graphic representation of the slight hypotonic gait data appears below in Figure 2. The FOB data were also suggestive of an increase in slight tremors at 1 and 10 mg/kg/day, though the incidence numbers, particularly at 1 mg/kg, were low.

RBC ChE activity was suppressed by statistically significant margins at the high dose on gd 20 and ppd 10 (Table III-15b). Suppression was noted on other measurement days as well, but didn’t achieve statistical significance. Brain ChE was statistically suppressed at the high dose on ppd 10, the only measurement day. The same trend was apparent for plasma ChE, though statistical significance was not indicated at any dose. Gross pathology did not reveal an

effect of carbaryl. The number of dead pups increased at the high dose (mean number / litter at ascending doses: 0.1, 0.1, 0.1, 0.3). Because it fell within the historical control range (0.0-0.9), the authors did not ascribe toxicologic significance to this effect. However, an effect of carbaryl on pup death could not be ruled out.

F_1 pups. There were no unambiguous effects of carbaryl in the F_1 pups, though mean motor activity counts for day 13 females were elevated at 10 mg/kg/day at each of the six measurement intervals (mean counts for all intervals at ascending doses: 67.1±75.6, 75.1±63.3, 52.1±55.8, 111.0±114.8). Though these failed to achieve statistical significance, their consistency at all measurement intervals suggested the possibility of a treatment effect. Even so, it is noted that wide variability in the individual data led to very large standard deviations, decreasing the robustness of this particular data set. These data were not considered sufficient to establish a LOEL value. Some brain morphometric measurements showed differences between control and high dose animals in both F_1 pups sacrificed on ppd 11 and F_1 adults sacrificed on ppd 70. However, as these results were inconsistent in degree and direction (*i.e.*, smaller or larger morphometric distances), they were also difficult to attribute unambiguously to carbaryl exposure.

F_1 adults. There were no clear effects of carbaryl in the F_1 adults.

The LOEL determination for maternal effects hinged on whether or not the incidence of FOB signs was sufficiently robust at 1 mg/kg to support values of regulatory significance. At 10 mg/kg, very clear body weight gain decrements, RBC and brain cholinesterase inhibition, and FOB signs (pinpoint pupils, slight tremors, slight ataxic gait and slight hypotonic gait) were present. These endpoints, in the absence of signs at 1 mg/kg, would set the maternal NOEL at 1 mg/kg. However, there was weaker evidence from the FOB data for effects - slight hypotonic gait in particular, but also slight tremors - at 1 mg/kg. Benchmark dose analysis of the slight hypotonic gait data produced an LED_{10} of 0.25 mg/kg (see section IV.1.a.). Both 1 mg/kg and 0.25 mg/kg were used to gauge the potential acute risk from exposure to carbaryl. The NOEL for developmental effects was set at the high dose of 10 mg/kg/day, with no LOEL established for developmental endpoints. It was nonetheless recognized that elevated motor activity counts at 10 mg/kg/day in day 13 F1 females may have resulted from carbaryl exposure.

This study was deemed acceptable according to FIFRA standards.

Table III-15a. Selected functional observational battery observations in F_0 females during the period of dosing with carbaryl (gd 6 - ppd 10), F_0 females (Robinson and Broxup [1997])

	Carbaryl dose (mg/kg/day)			
	Control	**0.1**	**1.0**	**10.0**
Slight hypotonic gait				
gd 6 [a, c]	6/23 (26.1) [b]	7/26 (26.9)	7/26 (26.9)	11/23 (47.8)
gd 9 [c]	7/26 (26.9)	2/26 (7.7)	10/26 (38.5)	11/26 (42.3)
gd 12 [c]	5/26 (19.2)	5/26 (19.2)	13/26 (50.0)*	11/26 (42.3)
gd 15 [c]	7/25 (28.0)	11/26 (42.3)	14/26 (53.8)	10/26 (38.5)
gd 18 [c]	5/25 (20.0)	10/26 (38.5)	11/26 (42.3)	15/26 (57.7)**
gd 20 [c]	9/25 (36.0)	5/26 (19.2)	11/26 (42.3)	13/26 (50.0)
ppd 4 [a]	3/21 (14.3)	3/23 (13.0)	7/24 (29.2)	4/24 (16.7)
ppd 7	5/21 (23.8)	6/23 (26.1)	9/24 (37.5)	8/24 (33.3)
ppd 11	8/21 (38.1)	5/23 (21.7)	6/24 (25.0)	7/24 (29.2)
ppd 13	4/21 (19.0)	5/23 (21.7)	3/24 (12.5)	6/24 (25.0)
ppd 21	1/21 (4.8)	1/23 (4.3)	3/24 (12.5)	6/24 (25.0)
Slight ataxic gait				
gd 6	0/23 (0)	0/26 (0)	0/26 (0)	2/23 (8.7)
gd 9	0/26 (0)	0/26 (0)	0/26 (0)	1/26 (3.8)
gd 12	0/26 (0)	0/26 (0)	0/26 (0)	2/26 (7.7)
gd 15	0/25 (0)	0/26 (0)	0/26 (0)	0/26 (0)
gd 18	0/25 (0)	0/26 (0)	0/26 (0)	0/26 (0)
gd 20	0/25 (0)	0/26 (0)	0/26 (0)	1/26 (3.8)
ppd 4	0/21 (0)	0/23 (0)	0/24 (0)	3/24 (12.5)
ppd 7	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
ppd 11	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
ppd 13	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
ppd 21	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
Slight tremors				
gd 6	1/23 (4.3)	2/26 (7.7)	2/26 (7.7)	5/23 (21.7)
gd 9	0/26 (0)	0/26 (0)	2/26 (7.7)	4/26 (15.4)
gd 12	0/26 (0)	1/26 (3.8)	0/26 (0)	3/26 (11.5)
gd 15	0/25 (0)	0/26 (0)	0/26 (0)	0/26 (0)
gd 18	0/25 (0)	0/26 (0)	1/26 (3.8)	4/26 (15.4)
gd 20	0/25 (0)	0/26 (0)	0/26 (0)	8/26 (30.8)***
ppd 4	0/21 (0)	0/23 (0)	1/24 (4.2)	1/24 (4.2)
ppd 7	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
ppd 11	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
ppd 13	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
ppd 21	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
Pinpoint pupils				
gd 6	0/23 (0)	1/26 (3.8)	0/26 (0)	9/23 (39.1)***
gd 9	0/26 (0)	0/26 (0)	0/26 (0)	4/26 (15.4)
gd 12	1/26 (3.8)	0/26 (0)	0/26 (0)	7/26 (26.9)*
gd 15	2/25 (8.0)	3/26 (11.5)	0/26 (0)	12/26 (46.2)***
gd 18	1/25 (4.0)	1/26 (3.8)	2/26 (7.7)	13/26 (50.0)***
gd 20	1/25 (4.0)	0/26 (0)	1/26 (3.8)	16/26 (61.5)***
ppd 4	0/21 (0)	1/23 (4.3)	0/24 (0)	6/24 (25.0)*
ppd 7	0/21 (0)	0/23 (0)	3/24 (12.5)	12/24 (50.0)***
ppd 11	0/21 (0)	1/23 (4.3)	0/24 (0)	0/24 (0)
ppd 13	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
ppd 21	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)

Moderate dilation of pupils				
gd 6	2/23 (9)	7/26 (27)	5/26 (19)	1/23 (4)
gd 9	4/26 (15)	5/26 (19)	2/26 (8)	1/26 (4) ɤ
gd 12	2/26 (8)	0/26 (0) ɤ	2/26 (8)	0/26 (0) ɤɤ
gd 15	2/25 (8)	4/26 (15)	2/26 (8)	0/26 (0) ɤɤ
gd 18	3/25 (12)	4/26 (15)	4/26 (15)	1/26 (4)
gd 20	7/25 (28)	10/26 (38)	5/26 (19)	0/26 (0) ɤɤ
ppd 4	0/21 (0)	1/23 (4)	1/24 (4)	0/24 (0)
ppd 7	3/21 (14)	6/21 (29)	4/24 (17)	5/24 (21)
ppd 11	2/21 (10)	4/23 (17)	4/24 (17)	8/24 (33)
ppd 13	1/21 (5)	2/23 (9)	2/24 (8)	2/24 (8)
ppd 21	1/21 (5)	3/23 (13)	3/24 (13)	1/24 (4)
Signs (per animal basis) [d]				
gd 6	6/23 (26.1)	7/26 (26.9)	7/26 (26.9)	16/23 (69.6)***
gd 9	7/26 (26.9)	2/26 (7.7)	10/26 (38.5)	12/26 (46.2)
gd 12	6/26 (23.1)	5/26 (19.2)	13/26 (50.0)*	14/26 (53.8)*
gd 15	8/25 (32.0)	12/26 (46.2)	14/26 (53.8)	12/26 (46.2)
gd 18	6/25 (24.0)	10/26 (38.5)	12/26 (46.2)	18/26 (69.2)**
gd 20	9/25 (36.0)	5/26 (19.2)	10/26 (38.5)	18/26 (69.2)*
ppd 4	3/21 (14.3)	3/23 (13.0)	7/24 (29.2)	11/24 (45.8)*
ppd 7	6/21 (28.6)	7/23 (30.4)	12/24 (50.0)	17/24 (70.8)**
ppd 11	8.21 (38.1)	5/23 (21.7)	6/24 (25.0)	7/24 (29.2)
ppd 13	4/21 (19.0)	5/23 (21.7)	3/24 (12.5)	6/24 (25.0)
ppd 21	1/21 (4.8)	3/23 (13.0)	3/24 (12.5)	6/24 (25.0)

* Fisher exact test, p<0.05; **Fisher exact test, p<0.01; ***Fisher exact test, p<0.005. These statistical tests were executed by the risk assessor.

ɤ Fisher exact test, p>0.95; ɤɤ Fisher exact test, p>0.99. These statistical tests were executed by the risk assessor.

[a] Abbreviations: gd, gestation day; ppd, *post partum* day

[b] Numbers in parentheses are the incidence rates expressed in percentages.

[c] Shirley's non-parametric test using incidences between gd 6 and gd 20 indicates the presence of statistically significant responses at 1 and 10 mg/kg/day for slight hypotonic gait.

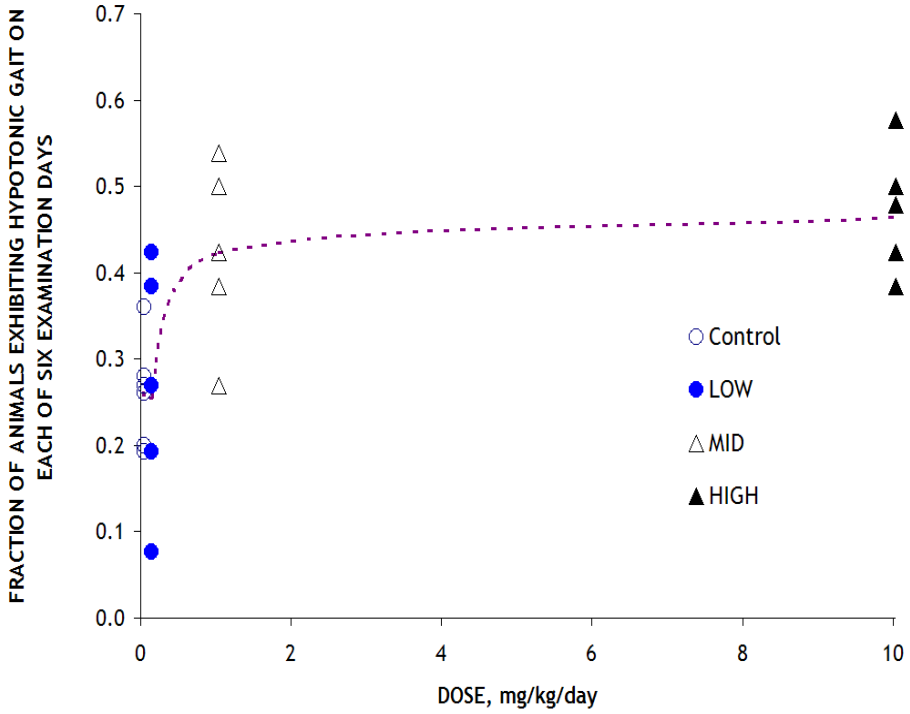
[d] Includes animals in which more than one sign was noted. Only positive signs were considered (*i.e.*, the decline in incidence of moderate dilation of pupils was not included).

Table III-15b. RBC, plasma and brain cholinesterase activities in F_0 female CD rats during the period of dosing with carbaryl (gd 6 - ppd 10) (Robinson and Broxup [1997])

	Carbaryl dose (mg/dg/day)			
	Control	**0.1**	**1.0**	**10.0**
RBC ChE (U/L)				
gd 6 [a]	988.3±204.71	990.0±129.77 (100.2)	913.5±272.84 (92.4)	800.0±194.49 (80.9)
gd 15	1127.0±172.19	1245.8±195.29 (110.5)	1203.5±249.12 (106.8)	1064.3±325.46 (94.4)
gd 20	1173.7±86.84	1171.7±91.94 (99.8)	1251.2±179.24 (106.6)	845.4±93.90** (72.0)
ppd 4 [a]	844.3±170.02	869.3±181.60 (103.0)	943.0±113.56 (111.7)	752.2±127.43 (89.1)
ppd 10	894.3±14.84	938.0±91.64 (104.9)	933.0±127.11 (104.3)	643.2±41.25** (71.9)
Plasma ChE (U/L)				
gd 6 [a]	844.8±287.11	964.4±209.26 (114.2)	902.0±333.78 (106.8)	697.8±240.35 (82.6)
gd 15	981.7±335.65	1097.5±207.81 (111.8)	1149.5±419.31 (117.1)	975.0±314.37 (99.3)
gd 20	1049.0±129.73	1134.2±212.91 (108.1)	1124.2±291.13 (107.2)	644.0±134.76 (61.4)
ppd 4 [a]	729.0±140.22	696.7±190.06 (95.6)	702.0±234.21 (96.3)	498.2±66.44 (68.3)
ppd 10	560.0±103.26	491.2±66.55 (87.7)	539.2±164.74 (96.3)	359.0±81.32 (64.1)
Brain ChE (U/g)				
ppd 10	5.9±0.04	6.2±0.22 (104.7)	5.8±0.16 (97.9)	3.4±0.58** (58.2)

[a] Abbreviations: gd, gestation day; ppd, *post partum* day
Parenthetical values represent percent of concurrent controls.

Figure 2. Scatter plot depicting the fraction of animals exhibiting slight hypotonic gait on each of six examination days[11]; the dotted line is a representation of the average response at the doses tested.



[11] Superimposition of data points resulted in less than six identifiable points / dose in this figure.

I. TOXICITY OF THE CARBARYL DEGRADATES AND METABOLITES

1. 1-Naphthol

Human exposure to 1-naphthol likely occurs through the metabolism of carbaryl or naphthalene. Exposure is also plausible through the use of this chemical in microscopy, as a coupler in cosmetic hair dyes, or in the manufacture of dyes and intermediates (CIR Expert Panel [1989]). Poole and Buckley (1989), citing a 1980 EPA TSCA review, stated that, "In humans a large ingestion of naphthol can cause nephritis, vomiting, circulatory collapse, anaemia, convulsions and death, and if sufficient quantities are absorbed through the skin, injury to the cornea and lens of the eye and also the kidney may occur". Reviews from two cosmetics industry panels (the Cosmetics Indgredient Review Panel [CIR Expert Panel [1989] and the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers [SCCNFP, 2001]) summarized the limited data available on the mammalian toxicity of 1-naphthol, using largely the same database of studies. As indicated by the citations below, much of the following information is derived from those reviews, with specific study references to be found within them. In addition, short TSCA (Toxic Substances Control Act) summaries were available. These summaries are quoted below along with their references.

Pharmacokinetics. Male mice receiving 1-naphthol by oral gavage (corn oil vehicle) showed a 24-hr elimination of 68% in the urine and 13% in the feces; the major metabolites were 1-naphthyl glucuronide and 1-naphthyl sulfate. Intraperitoneal injection of Sprague-Dawley rats with 7.5 µm/kg 1-naphthol (2-methoxyethanol vehicle) resulted in 83.5% urinary elimination / 16.5% tissue retention at 4 hr and 91.0% urinary elimination / 1.4% fecal elimination / 7.6% tissue retention at 48 hr. In a separate study, 1-naphthol labeled with ^{14}C at the 1-carbon was administered intraperitoneally to cats, pigs and rats at a dose of 25 mg/kg; at 24 hr, 91% of the radioactivity had been excreted in the urine of cats (98% sulfate conjugate,1.4% glucuronide conjugate), 81% in the pig (32% sulfate, 66% glucuronide) and 59% in the rat (53% sulfate, 47% glucuronide). Incubation of radiolabeled 1-naphthol with human blood for 24 hr resulted in the binding of 97.6% to plasma (92.8% of that in albumin, 3.6% in heavy lipoprotein and 3.6% in light lipoprotein fractions). In the same study, injection of mice with 1-naphthol, followed after 10 min by sacrifice and blood centrifuation showed 20-30% in the RBC fraction; the plasma fraction showed 43% associated with albumin and 43% with lipoproteins. A very limited study using three male volunteers determined that 1-naphthol contained in an ointment was rapidly absorbed. (CIR Expert Panel, 1989)

Acute oral toxicity. LD_{50}, rats: 2300 (1700-3300) mg/kg - study #1; 2590 mg/kg - study #2. (SCCNFP, 2001)

Poole and Buckley (1989), in the acute dosing section of a larger study (subchronic section below), treated two CD1 mice/sex/dose with 1-naphthol by gavage. The doses were 0-untreated control, 0-vehicle control (vehicle: propane-1,2-diol : water, 1:1), 0.5, 1 or 2 g/kg 1-naphthol. Survivors were observed for up to 2 weeks post dose. Sacrifice was followed by *post mortem* exam, blood analysis (clinical chemistry and hematology), and fixation of major organs for histolopathologic analysis.

All high dose mice were killed *in extremis* between 15 and 90 minutes after dosing. They exhibited tremors, abnormal respiration and collapse. All mid dose animals survived, exhibiting, then recovering from, subdued behavior and piloerection. Low dose animals also showed these signs (in addition to labored breathing); one low dose animal was killed *in extremis* 2 hr post dose, while the other three animals survived.

Histopathologic changes were noted as follows. Kidney: (1) both high dose males, one low dose male and both mid dose females exhibited "eosinophilic deposits in the lumen of the distal tubules and collecting ducts associated with degeneration of the distal tubular epithelia"; (2) one mid dose male and both mid dose females exhibited "papillary necrosis with an associated intravascular thrombosis"; (3) both mid dose females exhibited "marked dilatation of both cortical medullary tubules". Gut: (1) all but one of the mid and low dose mice exhibited "focal splitting of the squamous epithelium, which was generally associated with vascular congestion and an acute inflammatory cell infiltration"; (2) all high dose mice, one male and one female mouse and one low dose male exhibited "sloughing of the superficial epithelium of the glandular mucosa... generally, this change was associated with vascular congestion and an acute inflammatory cell infiltration". There were no effects noted on hematologic or clinical chemical parameters (though it is noted that blood was not obtained from those animals sacrificed *in extremis*).

An acute LOEL was set at the low dose of 0.5 g/kg, based on death and histopathologic changes in the kidneys and gut. An acute NOEL was not determined.

This study was considered to be supplemental.

TSCA submissions: "1-Naphthol (CAS # 90-15-3) was evaluated for acute oral toxicity. The test substance was administered by stomach intubation to non-fasted male albino Harlan-Wistar rats. The observed LD50 was 2.38 (1.56 to 3.65) g/kg, and 1.87 (1.27 to 2.76) g/kg for young and older adult rats, respectively. No further information was submitted.
[UNION CARBIDE CORP; Temik and Other Materials Miscellaneous Single Dose Peroral and Parenteral LD50 Assays and Some Joint Action Studies; 01/20/70; EPA No. FYI-OTS-0885-0443; Fiche No. OTS0000443-0]**UNREVIEWED**"

"1-Naphthol (CAS # 90-15-3) was evaluated for acute oral toxicity. The test substance was administered as a 50% solution in peanut oil. Rats receiving lethal doses suffered from diarrhea and died within 18 hours after treatment. Pathological examination indicated congestion and edema of the lungs; albumin in the kidney tubules; and superficial necrosis of the stomach. The approximate lethal dose (ALD) was calculated to be 1000 mg/kg.
[Letter to USEPA Regarding the Enclosed Acute and Chronic Oral Toxicity Studies on 1-Ethoxy-4-Nitrobenzene with Attachments (Sanitized); 11/27/91; EPA No. 86-920000378S; Fiche No. OTS0533716]**UNREVIEWED**"

Acute dermal toxicity. TSCA submission: "1-Naphthol (CAS # 90-15-3) was evaluated for acute dermal toxicity. The test substance was administered to 5 albino rabbits at a dosage of 10,000 mg/kg. No mortality and no signs of intoxication occurred. Dermal irritation consisted of moderate erythema and edema. Gross autopsy revealed no significant findings.
[Summary Results Concerning an Acute Oral LD50, Acute Eye Irritation, Primary Skin & Eye Irritation Indexes & 28-Day Subacute Feeding Studies with Cover Sheet & Letter (Sanitized); 00/00/00; EPA No. 86-920000514S; Fiche No. OTS0533803]**UNREVIEWED**"

Acute / sub-acute inhalation toxicity. Four adult dogs (splenectomized 4-8 yr prior) were exposed for four 7-10 min periods, 4 times/day, for 4 days to 3% 1-naphthol (deodorized kerosene vehicle). The study ran for 10 days. Other than the observation that one of the four dogs exhibited at least a doubling in the number of reticulocytes on days 7 and 10, there were no effects noted. (CIR Expert Panel, 1989)

Subchronic oral toxicity. "1-Naphthol orally administered to rats (20 males and 20 females)

for 12 weeks (5 times a week) showed that the dose of 20 mg/kg b.w./day (10 ml/kg) does not represent a toxic cumulative dose." (SCCNFP, 2001)

Poole and Buckley (1989), in the subchronic dosing section of their study (for the acute section, see above), treated five CD1 mice/sex/group with daily gavage doses for 30 consecutive days. The doses were 0-untreated control, 0-vehicle control (vehicle: propane-1,2-diol : water, 1:1), 50, 100 and 200 mg/kg. Sacrifice on day 31 was followed by *post mortem* exam, blood analysis (clinical chemistry and hematology), and fixation of major organs for histolopathologic analysis.

Two high dose males were sacrificed *in extremis* on study days 4 and 20, respectively. Both of these animals "showed evidence of focal mucosal erosion of the gandular stomach with some evidence of healing and peeling of the mucosa of the forestomach. The lesions were believed to have contributed to the poor clinical condition of the mice." A third high dose male also showed focal erosion of the glandular stomach, but survived. All females survived treatment, with none of the high dose animals showing gastric lesions.

While clear dose-related effects were not observed for clincal chemical parameters, hematologic analysis did reveal an apparent dose-related rise in white blood cell counts among females (at increasing doses the WBC counts in females were 7.64-untreated control, 6.24-treated control, 9.45, 10.5 and 12.2 x 10^9 / L) , though this was less clear in males (4.88-untreated control, 4.48-treated control, 7.13, 8.10, and 6.35 x 10^9 / L). The report claims that these increases were within the historical control range for the laboratory.

Body weight gains were suppressed at all doses, though a dose relation was not evident. Thus weight gains in control males and females was 4.9±2.3 g and 4.4±1.7 g, respectively, while in the combined dose groups they were 1.7±1.4 and 1.6±1.4 g.

A subchronic LOEL was set at the low dose 50 mg/kg, based on weight gain decrements and possible effects on female white blood cell counts. A subchronic NOEL was not determined.

This study was considered to be supplemental.

Subchronic dermal toxicity. "A formulation containing 1-naphthol (0.5%), mixed 1:1 with hydrogen peroxide, topically applied [1 hr/day] for 13 weeks (twice weekly) on abraded and intact skin of rabbit showed no evident toxic effect." (SCCNFP [2001])

Chronic toxicity and carcinogenicity dermal route. "One oxidative formulation (7403, mixed 1:1 with 6% hydrogen peroxide) containing 0.5% 1-naphthol was tested on Swiss Webster mice by [once weekly] dermal application (0.05 ml/cm^2 x 21 months). No adverse effects were reported." (SCCNFP, 2001). In addition, there was no evidence for carcinogenicity. (CIR Expert Panel [1989])

Irritation (skin). "The compound was applied to intact and abraded skin of rabbit at doses of 2.5% (0.5% aqueous gum tragacanth solution with 0.05% sodium sulphite, pH=7); it resulted not irritating [*sic*] after reading at 24 and 72 hours (primary irritation index = 0). No signs of irritancy were noted." (SCCNFP [2001])

Skin irritation was tested in guinea pigs with three lots of 1-naphthol applied as a 3% suspension, 0.5 ml per animal, to a shaved area of 1 in^2. Minor irritation was detected with two lots at 24 hr, but not at 48 or 72 hr. (CIR Expert Panel [1989])

"When applied to the skin of rabbits for 24 h, 500 mg of 1-naphthol caused severe irritation. Moderate irritation of the skin was observed when rabbits were treated with 550 mg 1-naphthol in open patches." (CIR Expert Panel [1989])

TSCA submission: "1-Naphthol (CAS # 90-15-3) was evaluated for primary dermal irritation. The test substance was administered at a dosage of 500 mg to the intact and abraded skin of 6 albino rabbits. Moderate to severe erythema and edema was noted after 72 hours (irritation score of 7.09/8.00).
[Summary Results Concerning an Acute Oral LD50, Acute Eye Irritation, Primary Skin & Eye Irritation Indexes & 28-Day Subacute Feeding Studies with Cover Sheet & Letter (Sanitized); 00/00/00; EPA No. 86-920000514S; Fiche No. OTS0533803]**UNREVIEWED**"

Irritation (mucous membranes). "The compound was instilled into one eye of 12 rabbits at concentrations of 0.5% - 1.5% - 2.0% - 2.5% w/v (0.5% in aqueous gum tragacanth with 0.05% sodium sulphite, 3 animals/dose) and the eyes were washed out 10 sec after treatment. The results (ocular reaction evaluated at 1 h and 1-2-3-4-7 days) showed the minimum irritant level, between 1.5% and 2.0%: positive reactions were observed in 2/3 of the rabbits at 2.0% w/v and 1/3 of the rabbits at 2.5% w/v." (SCCNFP [2001])

"When applied to the surface of rabbit eyes, 1-naphthol caused damage to the corneal epithelium at a grade of 9 on a scale of 1-10. 1-Naphthol, 1 mg, when instilled into the eyes of rabbits, caused severe irritation." (CIR Expert Panel [1989])

TSCA submission: "1-Naphthol (CAS # 90-15-3) was evaluated for primary eye irritation. The test substance was administered at a dosage of 100 mg to 6 albino rabbits. Slight to moderate effects of the cornea, iris, and conjunctivae were noted (irritation score of 61.7/110).
[Summary Results Concerning an Acute Oral LD50, Acute Eye Irritation, Primary Skin & Eye Irritation Indexes & 28-Day Subacute Feeding Studies with Cover Sheet & Letter (Sanitized); 00/00/00; EPA No. 86-920000514S; Fiche No. OTS0533803]**UNREVIEWED**"

Sensitization. "1-Naphthol (3% in water with 2.0% Natrosol, 2% Tween 80, 0.05% Sodium sulphite and 10% isopropanole) showed no allergic reaction in guinea pig by open epicutaneous method." (SCCNFP [2001])

"Sensitization was induced in 20 guinea pigs by simultaneously intradermal injections in the shoulder region of 0.1 ml of Freund's Complete Adjuvant (FCA), 0.1 ml 1-naphthol (0.1% in water) and a 1:1 mixture of test compound and 0.05 ml Adjuvant at day 0. The test compound was dermally applied (0.1% in water) 7 days later, under occlusion, on the injection site for 48 hours. 14 days later the guinea pigs were challenged by dermal application on the flank with 0.1% and 0.05% of 1-naphthol (aqueous solutions), under occlusion for 24 hours. The results evaluated after 24 and 48 hours of challenge showed that 1-naphthol was not a sensitiser in guinea pigs. Result: The sensitisation capacity was not properly assessed because the choice of concentration, for induction and challenge, may have been too low." (SCCNFP [2001])

Teratogenicity / embryotoxicity. "A formulation containing 1-naphthol (0.5%, 1:1 with hydrogen peroxide) was topically applied [2 ml/kg/day or 10 mg/kg/day] to the shaven skin of rats on day 1-4-7-10-13-16-19 of gestation. Only a significant reduction of the mean number of corpora lutea was observed between treated and two control groups (12.85 *vs.* 15.35 or 13.55). There was no evidence of any teratogenic or other adverse effect in the developing embryo / foetus." (SCCNFP [2001])

"25 female Sprague-Dawley Albino rats/group; Dosage 20, 40, 80 mg/kg bw. 1-Naphthol daily day 6 to 15 of gestation; Blank control (solvent); positive control 15 mg/kg/ vit. A; Acknowledged

methodologies. Results: At any dose level no treatment related effects. No maternal nor embryonic or foetal signs attributable to the test substance. In conclusion no maternal or embryo-toxicity, no incidence of embryo-lethality or growth retarding effects; no teratogenicity up to the highest tested dose of 80 mg/kg." (SCCNFP [2001])

Mutagenicity / genotoxicity. The following studies were summarized by the CIR Expert Panel, 1989:

- ♦ Nine *Salmonella* / Ames studies using various strains were negative. One study was positive in strain TA1538, with a maximal effect at 500 mg/plate in the presence of S9 microsomes (negative in three other strains). One study was positive in five strains in the absense of S9 microsomes.

- ♦ Two mutagenicity / DNA repair assays in various *E. Coli* strains, ±S9 microsomes, were negative.

- ♦ A Rec assay in *B. subtilis*, was positive in the absence of S9 and negative in the presence of S9.

- ♦ Micronucleus assays in rat and mouse bone marrow were negative.

- ♦ Examination of lymphocytes from men and women who had dyed their hair every 3-6 weeks for 11 months showed no effects on sister chromatid exchanges or chromosomal aberrations.

- ♦ Rat bone marrow cells were negative for chromosome aberrations.

- ♦ The mouse lymphoma cell line L5178Y did not show gene mutations upon *in vitro* exposure.

- ♦ Unscheduled DNA synthesis did not occur in rat hepatocytes in response to 1-naphthol exposure.

- ♦ An *in vivo* multigenerational Basc test in *Drosophila* was negative.

In the context of a discussion of genotoxicity, it should also be recalled that 1-naphthol, like carbaryl, induced an aberrant form of mitosis called c-mitosis that may reflect effects on mitotic spindle formation (Soderpalm-Berndes and Onfelt [1988] - see discussion above, section III.E.3.).

***In vitro* cytotoxicity**. 1-Naphthol was cytotoxic in several *in vitro* systems, including sarcoma BP 8 cells, chick embryo trachea organ cultures, rat primary hepatocytes, HeLa cells and human skin fibroblasts. (CIR Expert Panel [1989])

2. Methylamine

Methylamine is produced upon hydrolytic breakdown of carbaryl, which occurs under alkaline conditions. This compound is known for its irritant properties to eyes, nose and throat upon brief exposures to 20 - 100 ppm. Severe methylamine exposure may lead to pulmonary edema. The oral LD_{50} in rats is 100 - 200 mg/kg (Proctor *et al*, 1988). Shelby *et al.* (1987) demonstrated

positivity in the L5178Y mutagenicity assay. Gavage exposure of mice to 122 mg/kg methylamine for 5 days produced a statistically significant 27% drop in white blood cell counts (Keil *et al.* [1996]).

Regulatory limits. The OSHA PEL for methylamine is 10 ppm. The ACGIH TLV (TWA) is 5 ppm, with a STEL of 15 ppm.

IV. RISK ASSESSMENT

A. HAZARD IDENTIFICATION

1. Non-oncogenic effects

a. Acute oral toxicity

The most sensitive acute (or short-term) toxicologic endpoints for carbaryl were identified in the gavage developmental neurotoxicity study of Robinson and Broxup (1997). Because the putative effects at 1 mg/kg were not definitive, two acute regulatory values were derived from this study.

(1) A NOEL of **1 mg/kg**, based largely on clear, statistically significant FOB signs (slight hypotonic gait, slight ataxic gait, slight tremors and pinpoint pupils) observed at the high dose of 10 mg/kg, was established. Since those signs were present on the first day of dosing (gd 6), they were unmistakably acute in nature, though effects seen at any time during the FOB testing were arguably acute (see the discussion of carbaryl disposition in mammalian systems below). In addition, a statistically significant decrease in bodyweight gain at 10 mg/kg/day was noted at the first post dosing measurement on gd 9. This also represented an acute or near-acute effect, as the measurement was made only three days after the start of dosing. It is not known if the lowered cholinesterase activities observed at 10 mg/kg (statistically significant in RBCs at gd 20 and ppd 10, and in brain at ppd 10, the only day the brain enzyme was assayed) were acute or required several daily exposures. However, it is noted that RBC cholinesterase activities were suppressed by almost 20% ($p>0.05$) even at the first post dosing measurement on gd 6.

(2) A "lower bound on the effective dose at the 10% level" (LED_{10}) of **0.25 mg/kg** was obtained through benchmark dose (BMD) modeling applied to the slight hypotonic gait incidence data gathered during gestation. While these data were not as robust as the FOB and body weight data at 10 mg/kg used to support the 1 mg/kg NOEL designated in #1 above, they nonetheless represented a plausible reflection of cholinergic effects even at the mid dose of 1 mg/kg.

BMD is a method by which a threshold, or benchmark dose, is established for a toxicologic endpoint using mathematically fitted curves to model the data over most or all of the dose range. The benchmark response level for slight hypotonic gait was set to 10%, a value generally used by DPR to characterize mild toxicologic signs. Because of carbaryl's propensity for clearance from the rat system in less than 24 hours (Struble, 1994) and the relatively rapid decarbamylation reaction ($t_{0.5}$ = 40 min [*cf.*, Cranmer, 1985]), all of the FOB tests conducted during the gestation period were considered to represent separate, but equivalent, acute scenarios [12]. Consequently, the gestational data sets were combined to generate a normalized mean incidence rate, as noted in Table IV-1.

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[12] Post gestational exposures were not included in the analysis because the animals appeared less sensitive following the end of pregnancy.

Use of mean data in the BMD analysis was preferable to use of data from any day in isolation, as it minimized the random fluctuations noted in single day tests.

Initial attempts to model the full mean data set using the algorithms available in USEPA's BMD application version 1.3 were inappropriate because they underestimated responsiveness between 0.1 and 1 mg/kg, the dose-response region of primary interest. This was due to the pronounced leveling of the curve above 1 mg/kg (Figure 2), which led to underestimation of the slope between 0.1 and 1 mg/kg and resultant LED_{10} values higher than a putative effect level at 1 mg/kg. However, deletion of the top dose resulted in an appropriate curve fit using the probit algorithm (Appendix I). As noted, the resultant LED_{10} was 0.25 mg/kg (ED_{10} = 0.47 mg/kg).

Three acute toxicity studies from the same laboratory established low dose acute LOELs at 10 mg/kg in the rat, similar to the level at which there were overt clinical signs, body weight gain deficits and suppressed cholinesterase activities in the study of Robinson and Broxup (1997). These were considered supportive both of the 1 mg/kg NOEL and the 0.25 mg/kg LED_{10}.

(1) The acute gavage study of Brooks and Broxup (1995b) demonstrated clear inhibition of all cholinesterases (including brain cholinesterase) at this dose. For example, at 0.5 hr post dose, brain cholinesterase activities at 10, 50 and 125 mg/kg were 46%, 23% and 18% of concurrent controls, respectively, while female activities were 54%, 24% and 22% of controls. Benchmark dose analysis using the Hill algorithm of the male data yielded LED_{10} (ED_{10}) values of 0.61 (0.94) mg/kg. Marked inhibition was also seen for plasma cholinesterase in this study, though somewhat less inhibition occurred with RBC cholinesterase. Recovery was substantial by 24 hr.

(2) Brooks and Broxup (1995c) demonstrated a low dose rat acute gavage LOEL of 10 mg/kg based on cholinesterase inhibition at 1 hr post dose in a study designed to examine the time course of inhibition. In particular, brain cholinesterase was inhibited to 57%-73% of control activities at that time.

(3) A study of neurobehavioral and neuromorphologic effects of carbaryl after acute gavage exposure established a low dose LOEL of 10 mg/kg (Brooks *et al.*, 1995). This was based on a statistically significant reduction in motor activity counts in both sexes over a 60-minute period in both sexes on the day of dosing.

In a study from a separate laboratory, Moser (2007) demonstrated dose-dependent, statistically significant brain ChE inhibition in pnd11 rats at gavage doses as low as the lowest tested dose of 3 mg/kg. This resulted in a LED_{10} (ED_{10}) determination of 1.14 (1.46) mg/kg using benchmark dose methodology - essentially the same as the critical acute NOEL of 1 mg/kg and reasonably close to the LED_{10} of 0.25 mg/kg.

Support for the critical acute NOEL and LED_{10} designations also came from the rat 50-day neurotoxicity study (Desi *et al.*, 1974). Changes in maze performance, including faster goal attainment with fewer errors, were evident soon after the commencement of dietary exposure at 10 and 20 mg/kg/day carbaryl, the only doses tested. These early changes were probably acute in nature. They were followed some weeks later by other changes, including slower goal

attainment and more frequent errors, which probably represented responses to subchronic exposures.

Two older dog developmental toxicity studies from the open literature indicated additional effects at a similar dose range (Smalley *et al.,* 1968; Immings *et al.,* 1969). While these studies involved multiple dosing regimes, the possibility that the some of the effects were due to a single dose could not be discounted. These studies are discussed below in section IV.1.A.c.

Support for these regulatory values was also forthcoming in the acute inhalation toxicity study of Weinberg (2008), which established an LED_{10} of 5.5 mg/m^3 by BMD analysis from the results of a single 3-hr exposure. This was based on inhibition of brain cholinesterase activity at a low dose of 10 mg/m^3 (1.2 mg/kg, calculated using the default breathing rate of 0.96 m^3/kg) in females. The LED_{10} (ED_{10}) was equivalent to an internal dose of 0.66 (1.59) mg/kg, about halfway between the critical acute NOEL of 1 mg/kg and the LED_{10} of 0.25 mg/kg.

Table IV-1. Incidence of slight hypotonic gait during gestation in female CD rats, including mean values normalized to 26 animals (Robinson and Broxup, 1997)

	Carbaryl dose (mg/kg/day)			
	Control	**0.1**	**1.0**	**10.0**
Slight hypotonic gait				
gd 6 [a]	6/23 (26.1) [b]	7/26 (26.9)	7/26 (26.9)	11/23 (47.8)
gd 9	7/26 (26.9)	2/26 (7.7)	10/26 (38.5)	11/26 (42.3)
gd 12	5/26 (19.2)	5/26 (19.2)	13/26 (50.0)*	11/26 (42.3)
gd 15	7/25 (28.0)	11/26 (42.3)	14/26 (53.8)	10/26 (38.5)
gd 18	5/25 (20.0)	10/26 (38.5)	11/26 (42.3)	15/26 (57.7)**
gd 20	9/25 (36.0)	5/26 (19.2)	11/26 (42.3)	13/26 (50.0)
Slight hypotonic gait (mean)	6.8/26 (26.2)	6.7/27 (25.8)	11.0/26 (42.3)	12/1/26 (46.5)

* Fisher exact test, p<0.05; **Fisher exact test, p<0.01. These statistical tests were executed by the risk assessor.
[a] Abbreviation: gd, gestation day.
[b] Numbers in parentheses are the incidence rates expressed in percentages.

b. Chronic oral toxicity

The critical chronic oral LOEL was based on inhibition of brain cholinesterase activity at the low dose of 3.4 mg/kg/day (actually, 3.7 mg/kg/day in females and 3.4 mg/kg/day in males) in the 1-yr dog dietary study (Hamada [1987]). The brain cholinesterase data, which evidenced statistically significant 20% inhibition in females at 3.7 mg/kg/day compared to controls (14% non-statistically significant inhibition in males at 3.4 mg/kg/day; however, it was the latter dose that was used to determine the LOEL), was collected after 52 weeks of exposure. The RBC cholinesterase showed statistically significant inhibition at the mid and high doses (11.0 / 11.2 and 33.8 / 34.4 mg/kg/day, respectively) at all treatment intervals (weeks 5, 13, 26 and 52), while non-statistically significant inhibition was detected at the low dose (up to 14% in males at week 13 and 13% in females at week 5, the first measurement). Plasma cholinesterase activites were statistically suppressed in females at all doses for weeks 5, 13 and 26 (up to 23% at the low dose). Statistical significance in males occurred at the mid and high doses only.

The benchmark dose approach was employed to estimate a regulatory chronic LED_{10} value. The Hill algorithm for continuous data generated the most appropriate curve to fit the female Week 52 brain cholinesterase data. Neither the power nor polynomial algorithms generated comparable curves, either because AIC analysis indicated a higher value or because the overly complex curve shapes were considered unlikely to represent biological process. A 10% benchmark response rate was chosen in recognition of the fact that neither overt clinical signs nor histopathology were observed throughout the study, even at the high dose of ~34 mg/kg/day. Appendix II provides the details for the Hill algorithm calculations.

The critical chronic LED_{10} for brain cholinesterase inhibition in females using the Hill algorithm was **0.5 mg/kg/day** (ED_{10} = 1.7 mg/kg/day). This value will be used to evaluate the non-oncogenic risks from annual (*i.e.*, chronic) exposure to carbaryl.

c. Reproductive and developmental toxicity

Several epidemiologic studies indicate that carbaryl may have reproductive and/or developmental impacts (Wyrobek *et al.,* 1981; Savitz *et al.*, 1996; Meeker *et al.,* 2004a and 2004b; and Xia *et al.* 2005)). These are discussed below under Risk Appraisal, sections V.A.1.c. and V.A.1.d.. In addition, two studies in which pregnant beagle dogs were exposed to carbaryl throughout gestation (and until weaning in one study) indicated toxicologic effects both in the mothers and offspring. To the extent that some of these effects may have been acute (which was difficult to determine from the data as presented), they are considered supportive of the critical acute NOEL and LED_{10} based on the relatively low doses employed.

Smalley *et al.* (1968) observed an increase in dystocia - described as a “pattern” of difficult births (delayed delivery accompanied by restlessness, anorexia, fever, and the presence of a green-black, foul-smelling vaginal discharge; also, placental separation and atonic uterine musculature were evident in some cases) - in beagles exposed at a LOEL dose of 3.125 mg/kg/day. The number of dams with dystocia / number bred at 0, 3.125, 6.25, 12.5, 25 and 50 mg/kg/day were 0/16, 3/10, 3/10, 5/18, 3/9 and 3/8, respectively. In addition, the following observations were recorded in the Smalley study:

(1) One female from each of three dose groups (6.25, 25 and 50 mg/kg/day) sustained total fetal deaths, an observation which could not be dissociated from carbaryl exposure.

(2) While the mean pup weights were similar among dose groups at birth, the rate of pup weight gain in the combined dose groups was less than the control group by about 33%. Since pup weight data for individual dose groups was not reported, the implication was that the dose groups were not distinguishable in this regard.

(3) The percent of pups weaned decreased with dose (73%, 60%, 50%, 23%, 39%, 0%), though the cause of pup death was not reported. The association of effect with dose, particularly at the two lower doses (3.125 and 6.25 mg/kg/day), was not incontrovertible, as few animals were tested. But the consistency with the other observed effects made a relationship with carbaryl exposure possible.

(4) The number of litters containing pups with abnormalities - including “abdominal-thoracic fissures with varying degrees of intestinal agenesis and

displacement, varying degrees of brachygnathia, ecaudate pups (*i.e.*, without a tail], failure of skeletal formation, failure of liver development, and superfluous phalanges" [p. 392]) - increased with treatment above 3.125 mg/kg: 0/13, 0/7, 1/7, 3/16, 3/6, 1/2; historical controls: 3/313).

A developmental LOEL of 2 mg/kg/day was set in the study of Immings *et al.* (1969) based on a non-statistically significant increase in stillborn beagle dogs at that dose (Table III-12). Statistical significance was achieved at the mid and high doses when examined on a per-pup basis, though only at the mid dose when examined on a per-litter basis. Pup deaths, particularly those occurring after 24 hr *post partum*, also increased at 2 mg/kg, though there was concern that many of these animals were conceived during a period of elevated maternal illness. An effect at the litter level was not observed (see Table III-12 with its preceding discussion). Abnormalities - including umbilical hernia, cleft palate, fat-like mass in the heart, intussuception of the ileum into the colon, extravasation of blood in the myocardium and unilateral microphthalmia - were detected in the pups at the mid (5 mg/kg/day) and high doses (12.5 mg/kg/day). The co-incidence with the stillbirths and pup deaths was also attributed by the authors to maternal illness during the mating period. However, the proximity of the effective dose range in the two dog studies supported the possibility that there was an actual treatment effect.

d. Genotoxicity

With positive indications in one of five gene mutation studies, four of six chromosomal aberration studies and two of four DNA damage studies reviewed, carbaryl should be viewed as a potentially genotoxic compound. However, with the exception of one positive chomosome aberration study in *Allium cepa* (onion tree), a system that was of questionable relevance to mammalian systems, all of the positive studies were performed *in vitro*. In general, positive *in vitro* assays may be less relevant to the whole organism than positive *in vivo* results, though they may provide mechanistic insights in some cases.

One study demonstrated that nitrosocarbaryl could be produced from carbaryl and nitrite under acidic *in vitro* conditions. A separate study showed that nitrosocarbaryl caused chromosomal aberrations in Chinese hamster fibroblasts. Finally, one study in V79 Chinese hamster fibroblasts showed that, like carbaryl, the carbaryl metabolite $\propto$-naphthol was toxic and induced c-mitosis, an aberrant form of mitosis that may have reflected effects on mitotic spindle formation.

2. Oncogenicity

Overview. Carbaryl administered through the diet was oncogenic to both mice and rats in two-year studies. Dietary exposure in mice led to hemangiosarcomas and hemangiomas in both sexes, hepatocellular carcinomas and adenomas in females, and kidney tubular adenomas and carcinomas in males (Table III-7c) (Hamada [1993b]). Tumors did not appear within a 6-month time frame in p53 knockout mice (Chuzel [1999]), suggesting that, unlike the urethane positive control, carbaryl's effects in mice may not have involved the p53 gene product. However, carbaryl increased the lung tumor yield generated by two gavage exposures in mice to benzo[*a*]pyrene (Triolo *et al.* [1982]) and showed initiating capability in a standard mouse skin initiation-promotion assay (Shukla *et al.* [1992]).

Dietary exposure in rats led to carcinomas and papillomas of the urinary bladder in both sexes, hepatocellular adenomas in females and thyroid follicular cell adenomas and carcinomas in males (Table III-6c) (Hamada [1993a]). These were accompanied by possibly preneoplastic signs such as cellular hypertrophy or hyperplasia, squamous metaplasia, high mitotic index and/or atypia. In view of the positive genotoxicity tests (see previous section), it is premature to exclude genotoxicity as a possible driver in carbaryl-induced cancers in rodents, though direct evidence for genotoxically-driven tumors was lacking.

Mice. In view of the tendency of male mice to form hemangiosarcomas / hemangiomas at lower doses than were seen for the other tumors in this species, as well as the corroborating evidence from comparative benchmark dose analyses of all of the relevant mouse tumor data (see below; hemangiosarcoma / hemangioma, hepatocellular adenoma / carcinoma, kidney tubule cell adenoma / carcinoma), the human cancer risk was evaluated using the male mouse hemangiosarcoma / hemangioma data (Hamada [1993b]). Further support for use of this dataset came from knowledge that, while the high dose exceeded the maximum tolerated dose (MTD; this was based on early female deaths, body weight decrements and clinical signs - see section III.D.2. for a complete discussion), the mid and low doses did not. In addition, it should be recalled that carbaryl was genotoxic in several *in vitro* studies.

Hemangiosarcoma is defined as “a malignant tumor formed by proliferation of endothelial and fibroblastic tissue” (Dorland’s Medical Dictionary, 26th edition, p. 587). Hemangioma is defined as “a benign tumor made up of new-formed blood vessels” (Dorland’s, p. 587). The more encompassing term angiosarcoma includes “all lesions labeled hemangiosarcoma, lymphangiosarcoma, and malignant hemangiosarcoma, since it remains uncertain whether these lesions are derived from blood vascular or lymphatic endothelium, or perhaps from either” (Fletcher and McKee, 1992). The latter definition is mentioned in this context because it emphasizes the unsure cellular origin of this type of tumor.

Incidences of hemangiomas and hemagiosarcomas were combined as recommended by the National Toxicology Program (McConnell *et al.* [1986]). This reflects the conviction that the underlying tumorigenic process was similar for the benign and malignant types. A dose-responsive increase was noted in males (dose response of “at risk” males: 2/66, 6/66, 10/69* and 10/68* at 0, 14.73, 145.99 and 1248.93 mg/kg/day; *p<0.05; Table III-6c). Statistical significance in a Fisher pairwise test was achieved by the mid dose, though the increase between the control and low dose suggested that an effect was present even there. Females evinced a similar response, though it was manifest only at the highest dose and never attained pairwise statistical significance (dose response of “at risk” females: 3/63, 3/70, 4/66 and 9/61 at 0, 18.11, 180.86 and 1440.62 mg/kg/day [Table III-6c]). A USEPA-sponsored reanalysis of the the pathology slides generated very similar results with respect to all tumors noted, both in the mouse and rat (USEPA, 2002b). However, small changes with respect to incidence of hemangiosarcomas alone in male mice, from 2/66, 5/66, 9/69* and 7/69 (calculated from the incidences of this lesion among interim, unscheduled and terminal sacrifices) to 1/66, 6/66*, 8/69* and 8/68*, emphasized the likliehood that a tumorigenic response was present even at the low dose in the mouse study.

Fourteen separate algorithms available in USEPA’s version 1.3.2 benchmark dose (BMD) program were compared as potential models for the male vascular tumor data. The effectiveness of each model was assessed by consideration of the goodness of fit (through chi-squared residuals and p-values), analysis of deviance (through the AIC numbers), and

inspection of the curves for biological plausibility. Because the MTD was clearly exceeded at the high dose, resulting in a pharmacologic or metabolic profile of little relevance to humans exposed to low doses, it was excluded from the BMD analysis.

The quantal linear model emerged as the most appropriate for this dataset (see Appendix III for the full computer read-out of the male data). The resultant potency value for male mice, defined as the slope of the dose-risk relation, was 1.45×10^{-3} mg/kg/day^{-1} at the 95% upper bound on dose (*i.e.*, the LED) and 7.2×10^{-4} mg/kg/day^{-1} at the maximum likelihood estimate (*i.e.*, the ED). The LED / ED ratio was 2.01. The uncertainties inherent in this derivation of the male slope value are discussed in the Risk Appraisal section (V.A.2.).

In order to use the potency value to estimate risk to human populations, the mouse internal doses were converted to equivalent human doses and the human potency values calculated. Extrapolation of the mouse doses to humans was done by multiplying those doses by an interspecies scaling factor, using the ratio of animal-to-human bodyweight to the 1/4 power (US EPA [1992]): The mean wk. 53 male body weight was 38.4±2.2 g. Accordingly, the scaling factor was:

$$(BWt_A / BWt_H)^{0.25} = (0.0384 \text{ kg} / 70 \text{ kg})^{0.25} = 0.153$$

Thus the mean male mouse internal doses of 0, 14.73, 145.99 and 1248.93 mg/kg/day were converted to equivalent human doses of 0, 2.12, 21.02 and 179.85 mg/kg/day, from which the potency values were obtained (after deleting the high dose) by benchmark dose analysis using the quantal linear model. Thus the human oncogenic potency was **1.01×10^{-2} mg/kg/day^{-1}** at the 95% upper bound (based on the LED) and 5.03×10^{-3} mg/kg/day^{-1} at the maximum likelihood estimate (based on the ED). The 95% UB potency value was used to calculate the oncogenic risk from long term exposure to carbaryl (section V.C.2.).

Rats. Dietary exposure to carbaryl in rats resulted in transitional cell papillomas and transitional cell carcinomas of the urinary bladder (both sexes), hepatocellular adenomas (females), thyroid follicular cell adenomas and, possibly, follicular cell carcinomas (males) (Hamada, 1993a). These inductions were accompanied by hyperplasia, hypertrophy, squamous metaplasia, high mitotic index and / or atypia, which might be considered preneoplastic lesions.

With the possible exception of the liver tumors (see below), all tumor inductions occurred at the high dose (Table III-6c), which exceeded the MTD as determined by the large body weight decrements occurring at that dose (35% in males, 45% in females by study termination), as well as by the appearance of clinical signs and plasma, RBC and brain cholinesterase inhibition. Consequently, use of high dose data from this study for a quantitative risk evaluation was not indicated, as illness-inducing exposures may generate pharmacologic and/or metabolic profiles in the organism that are irrelevant to extended human exposures at low doses. There were, however, intimations of a rise in hepatocellular adenomas [13] in mid dose females, since the "at risk" rate was 1/64, 0/70, 3/69 and 7/68* (*$p<0.05$). In addition, a reanalysis of pathology slides conducted by the registrant and reported upon by US EPA (2002b) cited preneoplastic changes

[13] Adenoma: "a benign epithelial tumor in which the cells form recognizable glandular structures or in which the cells are clearly derived from glandular epithelium" (Dorland's Illustrated Medical Dictionary, 26th Edition, 1985; W.B. Saunders Company; p. 31).

at the mid and high doses in the week 53 interim sacrifice animals [14]. These included not only liver lesions (hepatocellular hypertrophy in mid and high dose males and in high dose females), but also transitional epithelial hyperplasia of the urinary bladder (mid and high dose males and high dose females) and hyperplasia of the cuboidal epithelium lining the papillary surface of the renal pelvis (mid and high dose males). US EPA (2002b) stated in the case of the urinary bladder that actual tumors may eventually have developed had the mid dose of 1500 ppm been somewhat higher:

> The MDT [*i.e.*, the mid dose tested of 1500 ppm] was judged to be below adquate for testing the carcinogenic potential of carbaryl. At this dose, there was no effect on body weight / body weight gain and only minor ChEI (less than 20% inhibition of plasma, RBC and brain ChE in males and females at week 53, except for 26% inhibition of RBC in females; at week 105, only female RBC and brain ChE were decreased (22% and 16%, respectively). The CARC [the Cancer Assessment Review Committee] noted that the MDT male rats had transitional cell hyperplasia of the bladder, a preneoplastic lesion, at the week 53 necropsy. If the dose had been adequate, bladder tumors seen at the HDT may have occurred at the MDT. (US EPA, 2002b; p. 10)

However, despite US EPA's contention that the mid dose did not exceed an MTD, the 9% and 18% body weight gain decrements in males and females, respectively, at that dose at 105 weeks (calculated from the weight gain data in Table III-6a) suggested that the mid dose was at least close to an MTD, as stated above in the summary of the study (section III.D.2.). In addition, the mid dose female hepatocellular adenoma incidence rate was similar to that in mid dose males, where there was no evidence of a dose-response relation for this tumor type (1/66, 1/67, 3/69, 1/67 at ascending doses). This may reflect the fact that the 4.3% incidence rate at that dose did not exceed the published historical control range of 0-6.3% for this tumor in female Sprague-Dawley rats (CPRC, 1994, quoted in USEPA, 2002b). These considerations raised the possibility that the mid dose incidence in females was either unrelated to carbaryl exposure or occurred at a dose too high for consideration in a quantitative risk assessment.

One further point should be made with regard to the rat study. As noted above, the US EPA argued that the mid dose bladder preneoplasias at week 53 found in the reanalysis may have developed into full tumors at a higher (but presumably still sub-MTD) dose. Their contention that the mid dose was below the MTD supported the qualitative relevance of the study to cancer risk assessment, particularly as the animals did develop bladder papillomas and carcinomas at the actual high dose. On the other hand, had the MTD been exceeded (as occurred at the high dose), it might have called into question the dosing regimen, and with it the relevance of the study to cancer risk assessment. DPR's view is that, while an MTD may have been approached, the multiplicity of tumors at the high dose combined with the presence of preneoplasias and the suggestion of an effect on hepatocellular adenomas at the mid dose lended support to the quantitative potency analysis carried out in the mouse.

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[14] These changes were not noted in the original report of Hamada, 1993a. Also, the histopathology data from the terminal animals did not change significantly between the reports.

B. DIETARY EXPOSURE ASSESSMENT

1. Introduction

Under the California Food Safety Act (AB-2161; Bronzan and Jones [1989]), the Department of Pesticide Regulation conducts acute and chronic dietary exposure assessments to evaluate the risk of human exposure to a pesticide in food. Two separate approaches are used to estimate the risk: (1) risk is determined for the total dietary exposure based on measured residue levels on all commodities with established federal tolerances, and (2) risk is estimated for exposure to an individual commodity at the tolerance level (see section VI. Tolerance Assessment).

Dietary exposure is the product of the amount of food that is consumed and the concentration of the pesticide residue in that food. The total exposure in an individual's diet for a defined time period is the sum of exposure from all foods consumed within that period, in various forms and as ingredients in processed food items.

Two distinct pieces of information are required to assess dietary exposure: (1) the amount of the pesticide residue in food, and (2) the food consumption (including drinking water). For estimating the acute exposure either the highest residue values at or below the tolerance or the distribution of residues are considered. In contrast, for chronic exposure the mean residue values are considered. Acute exposure is calculated on a per-user basis, which includes in the distribution of exposures only the days of survey that at least one commodity with potential pesticide residues is consumed. Chronic exposure to pesticides is generally calculated using per-capita mean consumption estimates.

2. Consumption data and dietary exposure

The Dietary Exposure Evaluation Model (DEEM-FCID®, version 2.03; Exponent Inc., http://www.exponent.com/home.html) was used as the dietary exposure software in this analysis. The food / drinking water consumption pattern was based on data generated by the United States Department of Agriculture (USDA) through its 1994-1998 Continuing Survey of Food Intake by Individuals (CSFII). The 1994-1998 dataset included the 1994-1996 food consumption survey along with the 1998 Supplemental Children's Survey (CSFII 1998). Risk estimates, expressed as margins of exposure (MOEs), were provided for the average US population and 18 selected population subgroups. These subgroups were defined by geographic regions, gender, ethnicity or age, and included infants (nursing and non-nursing) and children.

For acute exposure estimates, one-day consumption data comprised all of the several dozen commodities that carry tolerances for carbaryl. These include raw agricultural commdities, meat, dairy and drinking water (see Table IV-2) [15]. In the initial analysis, the consumption of each commodity by each member of a population subgroup was multiplied by a single residue value (point estimate) to generate a deterministic risk assessment. This single residue value was either the highest measured or, in cases in which no residues were detected, the limit of detection (LOD). In cases in which more than one LOD was used, as occasionally occurred when there were data for more than one year, the highest LOD was chosen.

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[15] In actuality, the DEEM-FCID analysis considered 350 food items, all of which were derived from the tolerances listed in the Federal Register (Volume 73, Number 176 - Sept. 10, 2008).

In order to refine the acute assessment toward a more representative probability of populational exposure, the entire range of measured residue levels was then considered in a distributional (Monte Carlo) analysis. One-half of the LOD value was assigned to those samples in the residue data file not yielding detectable residue levels (*i.e.*, were below the LOD). In one case - olives - residue data came from a field trial in which all of the twelve samples registered detections. These were used to construct an artificial RDF using a percent crop treated value of 2% and setting all values except for the measured residue values to the LOD.

For chronic exposure estimates, the average food consumption of each population subgroup was multiplied by the mean residue value. The dosage estimates for both acute and chronic exposure were expressed in units of ppm (µg/kg/day).

3. Exposure to carbaryl and 1-naphthol in food

Residue levels of carbaryl were determined for all food items with carbaryl tolerances, as indicated above. The tolerance values were published in the Federal Register (Volume 73, Number 176 - Sept. 10, 2008). Because the carbaryl metabolite 1-naphthol is included in the tolerances for all commodities except dillweed, an accounting of possible risks from exposure to that compound was called for. The PDP system included 1-naphthol in its measurements for 2004-2007. Far fewer measurements were made in the previous years, making the data from those years of limited value.

In 2004, carbaryl residues were detected in 396 of the 10,273 samples tested under PDP (3.9%), while 1-naphthol was detected in 26 of 1820 samples tested (1.4%). In 2005, carbaryl residues were detected in 343 of the 10,127 samples tested under PDP (3.4%), while 1-naphthol was detected in 32 of the 2796 samples tested (1.1%). In all cases in which 1-naphthol was detected during this 2-year span, carbaryl was also detected, though the reverse was not the case. This meant that the conditions that were likely to preserve naphthol in a given commodity also preserved carbaryl.

In most instances in which both compounds were detected in 2004 and 2005, the parent compound was present at higher levels than the degradate. Thus in 2004, for 23 of the 26 dual detections the naphthol / carbaryl ratio was 0.37±0.22. For 3 of the 26 dual detections, naphthol residues were higher than carbaryl (by 1.10-fold, 5.67-fold and 24.00-fold). In 2005, 24 of the 32 dual detections showed higher carbaryl residues than naphthol, with a naphthol / carbaryl ratio of 0.50±0.25; eight of the 32 dual detections showed higher naphthol residues than carbaryl, with a ratio of 2.16±1.52. In 2005, four additional measurements were made in pork products, all of which showed serious over-tolerances for both carbaryl and 1-naphthol. The naphthol-to-carbaryl ratio for those samples was 1.33±1.05.

Though it is true that about two-thirds of the carbaryl-positive samples did not contain 1-naphthol at detectable levels, the variegated nature of the combined residue data renders impossible valid generalizations of the relative residues of the parent compound and degradate. All that can be said is that if the assumption is made that the two compounds are of equivalent toxicity, the risks estimated when only carbaryl exposure is considered may be lower than the "actual" risk.

However, potential residues of the carbaryl metabolite 1-naphthol were *not* included in this analysis for the following reasons:

1) The acute oral toxicity of 1-naphthol was substantially less than that for carbaryl. The acute LD_{50} for 1-naphthol in rats was 2300-2590 mg/kg (SCCNFP [2001]), compared to LD_{50}s for carbaryl of 233-840 mg/kg in the same species (section III.B.3. above).

2) The only available acute LOEL for 1-naphthol, 0.5 g/kg, based on death and histopathologic changes in the kidneys and gut of rats (Poole and Buckley [1989]), was substantially higher than the critical acute LED_{10} of 0.25 mg/kg for carbaryl, which was based on cholinergic signs and symptoms at a LOEL of 1 mg/kg (Robinson and Broxup [1997]).

3) Subchronic oral toxicity was also observed at higher doses for 1-naphthol than for carbaryl. Poole and Buckley (1989) established an oral subchronic LOEL of 50 mg/kg/day for 1-naphthol in rats, based on weight gain decrements and possible effects on white blood cell counts in females. While a critical subchronic NOEL or LED_{10} was not established in this assessment, and LED_{10} of 0.5 mg/kg/day was noted based on dose-dependent inhibition of brain cholinesterase activity starting at a low dose of 3.4 mg/kg/day in the 1-yr dog dietary study of Hamada (1987).

4) As 1-naphthol results from the decarbamylation of carbaryl, the likelihood that it is an effective cholinesterase inhibitor is low. This is an important consideration in view of the high probability that both the acute and chronic risk estimates were based on cholinesterase inhibition. It should be noted, however, that the mechanism of carbaryl-mediated carcinogenesis is not understood. If 1-naphthol is carcinogenic, and if it exerts its influence at the same dose as carbaryl, the oncogenic risk estimate obtained by considering carbaryl alone may be understated.

4. Residue data sources

The residue data for carbaryl used in this assessment were derived overwhelmingly from the USDA's Pesticide Data Program (PDP). Data collected by the Food and Drug Administration were used for blueberries and raspberries. Field trial residue studies submitted by carbaryl registrants to support tolerances were used for olives, olive oil, sunflower seeds and sunflower oil.

The USDA Pesticide Data Program (www.ams.usda.gov/science/pdp/download.htm). The PDP was specifically designed to generate pesticide residue data for risk assessments. Since 1991, PDP data have been collected annually from ten states (including California) at produce markets and chain store distribution centers close to the consumer level. As described in the Annual Summary of the Pesticide Data Program for the 2007 calendar year, "PDP sampling procedures were designed to capture residues in the food supply as close as possible to the time of consumption. PDP concentrates its efforts to provide realistic pesticide residue data on foods that are most often consumed by infants and children and incorporates recommendations made in 1993 by the National Academy of Sceinces (NAS) in its report 'Pesticides in the Diets of Infants and Children.'" The current assessment utilizes carbaryl residue data collected mostly between 2004 and 2007. Earlier PDP data were used in cases where more recent data were not available.

PDP data were available for many of the commodities with established tolerances for carbaryl (Table IV-2). These were split in the DEEM-FCID® analysis into 350 total food items, eleven of which were again split into subcategories ("food forms") for both the acute and chronic analyses due to the availability of processing data on those forms and the conviction, borne out in preliminary DEEM-FCID runs, that the data on those relatively highly-consumed commodities would materially affect the calculated exposure. With the exception of three commodities - corn syrup, soybeans and finished drinking water - all of the PDP-based samples were collected in California. This resulted in risk estimates that bore particular relevance to California consumers.

For the Tier 2 / point estimate analysis, appropriate representative commodities ("surrogates") originating from the same crop group were used in all cases in which residue data on a given commodity were not available (see crop group tables, 40CFR 180.41).The high point estimate values were assigned to such commodities from monitored surrogates within the same crop group (Table IV-2) [16]. For the Tier 3 / distributional analysis, the decision to use a surrogate was based on examination of the Critical Exposure Commodity (CEC) report for the Tier 2 and preliminary Tier 3 analyses (see below). Where the CEC report indicated that there was sufficient consumption of a particular commodity to make a potentially substantial contribution to overall exposure (*i.e.*, greater than 5% of the total exposure), surrogate distribution data were used. In all such Tier 3 cases, the crop treated value was set to 100%. The commodity / surrogate pairs used in the Tier 3 assessment were as follows: *crop group 5B* - turnip greens / collard; *crop group 9A* - honeydew melon / cantaloupe; *crop group 11* - pear juice / pear; *crop group 12* - apricot / nectarine; apricot juice / nectarine; dried apricot / nectarine; *crop group 13A* - raspberry juice / raspberry, blackberry juice / blackberry; *crop group O* - strawberry juice / strawberry.

Carbaryl residues were detected in at least one sample from the following commodities under PDP (except where another source is indicated): *crop groups 1A and 1B* - none; *crop groups 1C and 1D* - frozen potato, sweet potato; *crop group 2* - none; *crop group 4A* - lettuce, fresh spinach; *crop group 4B* - celery; *crop group 5A* - broccoli; *crop group 5B* - collard, kale; *crop group 6* - none; *crop group 6A* - bean (snap/succulent/frozen), pea (fresh/frozen/canned); *crop group 6B* - none; *crop group 8* - eggplant, sweet bell pepper, tomato paste; *crop group 9A* - cantaloupe, watermelon; *crop group 9B* - cucumber, summer squash; *crop group 10* - grapefruit, orange, orange juice; *crop group 11* - apple, apple juice, apple sauce, pear; *crop group 12* - cherry, nectarine, peach, plum (prune/fresh), plum (prune/dried); *crop group 13A* - raspberry (FDA); *crop group 13B* - blueberry (FDA); *crop group 14* - none; *crop group 15* - rice; *crop group 19A* - none; *crop group 20* - sunflower seed (field trial); *crop group O* - asparagus, banana, cranberry, grape, grape juice, olive (field trial), olive oil (field trial) onion, pineapple, strawberry, drinking water ("finished"); *crop group M* - pork fat, pork muscle; *crop group D* - milk, heavy cream.

Carbaryl residues were not detected under PDP for the following commodities, necessitating use in those cases of the LOD for the point estimate determinations: *crop groups 1A and 1B* -

[16] Walnuts, for which no PDP data were available, presented a unique case. The Tier 2 point estimate for walnuts was taken from the almond point estimate value, as indicated in the surrogate methodology detailed here. However, the tolerance of 1 ppm for walnuts was 10-fold that of almonds (0.1 ppm), suggesting that the almond residue value may have less relevance to walnuts than surrogates used for other commodities in this assessment, all of which bore the same tolerance as those commodities.

carrot; *crop groups 1C and 1D* - potato, *crop group 2* - none; *crop group 4A* - canned spinach; *crop group 4B* - none; *crop group 5A* - none; *crop group 5B* - none; *crop group 6* - soybean; *crop group 6A* - beans (snap/succulent/canned); *crop group 6B* - none; *crop group 8* - tomato; *crop group 9A* - none; *crop group 9B* - winter squash; *crop group 10* - none; *crop group 11* - none; *crop group 12* - none; *crop group 13A* - none; *crop group 13B* - none; *crop group 14* - almond; *crop group 15* - sweet corn, corn syrup, wheat, wheat flour; *crop group 19A* - none; *crop group 20* - sunflower oil (field trial); *crop group O* - peanut butter, bottled water; *crop group M* - beef fat, beef liver, beef muscle; *crop group D* - none.

FDA monitoring program. The Food and Drug Administration monitors pesticide residues in imported foods and in domestic foods shipped *via* interstate commerce. FDA also collects incidence and residue level data on particular commodity / pesticide pairings. These functions are related to its tolerance enforcement mandate. Though some processed commodities are examined, FDA generally samples unwashed whole raw agricultural commodities, unlike the PDP program. Domestic sampling is executed as close to the point of production as possible, while sampling of imports occurs at the point of entry into the USA. Raspberry and blueberry residue data, which was supplied to DPR by the USEPA, originated with the FDA monitoring program.

Field trial data. Field trial studies are submitted by pesticide registrants to support the setting of tolerances. Because these studies are usually conducted under the maximum application rates for the proposed label, with sampling carried out on the treated commodities, field trial data are not preferable to PDP or FDA data in the generation of dietary exposure analyses. In the current assessment, field trial data were used only in cases where data from the other sources were not available: sunflower seeds (Robinson [1995a]), sunflower oil (Robinson [1995b]) olives (Macy and Lee [1995])[17] and olive oil (point estimate value supplied directly by the USEPA [USEPA, 2007]).

Finally, one commodity with a tolerance, oysters, had no entry in the DEEM program and thus was omitted from the analysis. While it was not expected that carbaryl exposure from oysters would comprise a substantial part of the total dietary exposure to carbaryl, the absence of this commodity from DEEM was, at the very least, a possible source of underestimation of that exposure.

5. Acute exposure

DPR uses a tiered approach to estimate acute dietary exposure to pesticides (DPR [2002]). Each succeeding tier refines the residue values assigned to commodities in the preceding tier. Tier 1 employs the tolerance value for each commodity, while Tier 2 is based on the highest residue value or limit of detection (LOD) for those same commodities. Tiers 1 and 2 represent "deterministic" approaches to exposure assessment. Tier 3 comprises the distributional (Monte Carlo) approach, by which the measured *distribution* of the residue values for a given commodity, rather than a single point value, is taken into account (all tiers consider the distribution of consumption in the various subpopulations). As is clearly evident for carbaryl, the Tier 3 level of refinement was necessary to generate a realistic picture of the acute dietary

[17] In the case of olives, an artificial RDF file was constructed assuming a total sample number of 600. The 12 residue detect samples (*i.e.*, every sample) along with the 2% crop treated value reported by the USEPA (USEPA, 2007) were also used.

exposure and potential risk. The results of the tiered approach are summarized in the following paragraphs.

Tier 1. Setting commodity residues at tolerance levels resulted in total exposure estimates substantially higher than the level considered as health protective for all examined subpopulations based on the calculation of Margin of Exposure (MOE = LED_{10} / exposure concentration) (data not shown). An MOE of 100 or greater is considered health protective in cases such as this one in which the toxicity data come from experiments in laboratory animals. As it is extremely unlikely that all commodities within an eating occasion would contain residues at the federal tolerance level, refinement of the exposure estimates in at the Tier 2 level was necessary.

Tier 2. Tier 2 utilized the highest of the measured residue values or LOD. In practice, this required the following assumptions: (a) all consumed foods contained the highest reported residue below the tolerance, (b) pesticide residues below the LOD were equal to the LOD, (c) all crops with tolerances were treated with the pesticide, and (d) residue concentrations did not vary from the time of sampling to the time of consumption.

Table IV-2 provides the residue values used in Tier 2 for carbaryl. As noted above, PDP data were used for the bulk of the commodities bearing tolerances, with field trial data used for olives, olive oil, sunflower seeds and sunflower oil, and FDA data used for raspberries and blueberries. With one exception, either the highest measured residue value or the highest LOD (*i.e.*, when no residue was detected) was used as the final point estimate. In the case of cactus, for which no residue data were available from PDP, FDA or field trials, the highest of all of the LODs for any commodity used in DEEM, 0.025 ppm, was substituted for a point estimate. Oysters, for which a tolerance exists, were excluded from the analysis because DEEM-FCID® had no category for that item.

Changes in food hydration state or processing will also alter residue concentrations from those measured in the raw commodities. The following processing / dehydration multipliers were used as they appear in the USEPA Revised dietary exposure analysis on carbaryl (USEPA [2007]) and entered into the DEEM-FCID® Acute Module (version 2.03) as "adjustment factor 2": apples - juice (used in this assessment for pear juice but not apple juice, as PDP data were available on the latter commodity): 0.37; apples - dried (used for apples and pears): 2.58; cabbage - cooking (used for cabbage, brussels sprouts and kohlrabi): 0.1; lemon peel: 1.16; orange peel: 1.27; corn grain - corn oil: 0.25; olives - olive oil: 0.81; okra - cooking: 0.66; okra - cooking/steaming: 0.18 (used for frying and non-specific preparation); peas - cooking: 0.15; peanuts - peanut oil: 0.29; pineapple - pineapple juice and pineapple peeled fruit: 0.54; plums - dried: 0.15; potatoes - dried: 0.4; potatoes - fried: 0.04; potatoes - baked: 1.2; potatoes - boiled: 2.5; rice - polished (white): 0.03; rice - bran: 0.4; soybean - oil: 0.005; sugarbeets (used for sugar and molassess): 0.04; tomatoes - puree (used for paste): 0.65; tomatoes - juice: 0.52; tomatoes - dried: 0.52; wheat - germ: 0.65; wheat - bran: 1.03.

In addition, default processing factors from DEEM® (version 7.6) were included in this analysis, as follows: apricots - dried: 6.0; bananas - dried: 3.9; beef - dried: 1.92; cherries - juice: 1.5; corn grain / sugar, high fructose corn syrup (used for corn syrup) - 1.5; cranberries - juice: 1.1; grapefruit - juice: 2.1; lemon - juice: 2.0; lime - juice: 2.0; peach - dried: 7.0; pineapple - dried: 5.0; plum / prune - juice: 1.4; potato - flour: 6.5; tangerine - juice: 2.3.

In general, the highest exposures were predicted for infants and young children (1-2 yr and 3-5 yr age groups in particular). Lower exposures were predicted for adults. Ethnicity and geographic localization were of somewhat less importance than age for the Tier 2 analysis. Table IV-3 contains the point estimate exposures at the 95th and 99th user-day percentiles (defined as the estimated percentile of user-days falling below the calculated exposure).

The acute Critical Exposure Commodity (CEC) analysis identified blueberries, peaches, apricots, apples, asparagus, olives, grapes and strawberries as making substantial (i.e., >5%) contributions to the acute dietary exposure of the highest exposure groups (infants, children 1-2 yr and children 3-5 yr) under Tier 2. The high contributing commodities in the US population as a whole included apples, asparagus and blueberries. For a complete Tier 2 contributor analysis, see Appendix VI-3.

Tier 3. Due to the low MOEs generated in the Tier 2 analysis (see section IV.C.5.a. below), a distributional (Monte Carlo) analysis was initiated as the next refining step of the acute dietary exposure assessment. This approach combined pesticide residue and commodity consumption to produce a distribution of potential exposures for the selected subpopulations. These exposure estimates, shown in Table IV-3, also incorporated information on the percentage of the crop that was treated with carbaryl (PCT), so that the actual use pattern for that crop was reflected in the exposure patterns generated. PCT data for each commodity were taken directly from USEPA's dietary analysis (USEPA, 2007), which in turn obtained the data from their own Biological and Economical Analysis Division (BEAD). Several of the commodities were considered to be "blended", *i.e.*, they contained contributions from many different harvests. PCT was set to 100% for these commodities because it was assumed that there were potential residues in all samples [18]. Included in the blended category were soybeans, tomato paste, orange juice, apple juice, apple sauce, grape juice, peanut butter, drinking water, bottled water, milk and heavy cream. PCT was also set to 100% for imported commodities (bananas and pineapples) and for all meat products.

Residue data files (RDFs) were constructed for all commodities for which there were adequate data. Such files consisted of all of the residue values for that commodity, along with the total number of samples assayed. The proportion of that total expected to have no residue at all was calculated using the PCT value, with the remaining samples set to half of the LOD. Thus:

$$\#\ @\ 0.5\text{xLOD} = (\text{total}\ \#) - (\#\ @\ \text{zero} + \#\ \text{with residue detections})$$

As with the point estimates, the Monte Carlo estimates were highest for infants (particularly non-nursing infants <1 yr) and young children in the 1-2 yr age group when assessing the 99.9th percentile. At the 95th percentile, the "non-hispanic / non-white / non-black" population (assumed to be Asian) was also among the more highly exposed. The least exposed populations tended to be older youths and adults.

The acute Critical Exposure Commodity (CEC) analysis identified cherries, rice, blueberries, peaches, pineapples, and plums as major contributors (*i.e.*, >5%) for the infant and children 1-2 yr subpopulations. For non-hispanic / non-white / non-black subpopulation, the major

[18] In other words, inclusion of percent crop treated data allowed "non-detect" samples to be set either at 0 mg/kg or at the LOD. This is explained in the calculation that appears in the next paragraph.

contributors were chinese cabbage and mustard greens. For the US population as a whole, cherries and plums were the major contributors. For a complete contributor accounting in the Monte Carlo analysis, see Appendix VI-5.

6. Chronic exposure

For estimates of chronic exposure, the average value of all pesticide residues detected on a commodity was multiplied by the average annual consumption determined for each subpopulation. The population average daily consumption distribution was used to reflect the consumption patterns of individuals over time. Residue levels below the LOD were set at 1/2 of that limit. PCT adjustments were not made for any commodity in the chronic analysis because the MOEs calculated assuming 100% PCT were greater than 100, obviating the need for further refinement. Even so, estimated average PCT values from USEPA's Dietary Risk Assesment on Carbaryl (USEPA [2007]) were included in Table IV-2 (along with the calculated chronic residue levels) for possible later use. As with the acute analysis, PDP was the predominant data source in the chronic analysis, except in cases where the data were based on field trials or FDA data. Calculations for oncogenicity using the 95% upper bound on the dose-tumor slope also used the chronic exposure data.

The DEEM-FCID®-generated chronic exposure estimates appear in Table IV-4. Children 1-2 yr exhibited the highest exposures, followed by non-nursing infants <1 yr and all infants. Exposure for the US population as a whole was notably lower. Inspection of the CEC analysis indicates that stone fruits (apricots in particular) were the major contributors to chronic carbaryl exposure for non-nursing infants <1 yr. For children 1-2 yr the major contributors were stone fruits and olives, while for all infants they were stone fruits. Olives were the main contributor for the US population as a whole.

Table IV-2. Anticipated **Carbaryl** Residues for Acute and Chronic Dietary Exposure Assessment - arrangement by crop group

Commodity	Source of data	Toler. (ppm)	detected samples / total samples	Detected residues (ppm)	LOD range (ppm)	PCT max - avg [a]	Acute residue (ppm) Pt. estimate [b]	Acute residue (ppm) Monte Carlo [c]	Chronic residue (ppm) [d]
Crop groups 1A and 1AB - Root vegetables									
Carrot	PDP, 2007 PDP, 2006	.5	0 / 744 0 / 744	n/a	.002 - .006	6% - 4%	0.006	RDF1	0.001
Beets, garden (root) Beets, sugar (root) Burdock Celeriac Chicory root Ginseng Horseradish Parsley (turnip root) Parsnip Radish Radish (Oriental) Rutabaga Salsify (root) Turnip (root)	PDP, 2007 PDP, 2006	2	0 / 744 0 / 744	n/a	.002 - .006	100% - 100%	0.006 (carrot)		
Crop group 1CD - Tuber and corm vegetables									
Potato	PDP, 2002 PDP, 2001 PDP, 2000	2	0 / 370 0 / 733 0 / 369	n/a n/a n/a	.008 .008 .008	3% - 2%	0.008	RDF2	0.004
Potato (frozen)	PDP, 2007 PDP, 2006	2	5 / 800 0 / 744	.002 n/a	.001 - .002	5% - <1%	0.002	RDF3	0.001
Sweet potato	PDP, 2004 PDP, 2003	0.2	0 / 653 1 / 734	n/a .013	.002 .002 - .008	38% - 17%	0.013	RDF4	0.002522

Arrowroot Artichoke, Jerusal. Cassava Dasheen, corm Ginger Tanier Tumeric, corm Yam, true Yam bean	PDP, 2004 PDP, 2003	0.2	0 / 653 1 / 734	n/a .013	.002 .002 - .008	100% - 100%	0.013 (sweet potato)		
Crop group 2 - Leaves of root and tuber vegetables									
Beets, garden, top Chicory, top Dasheen leaf Radish top Radish, Oriental top Salsify, top	PDP, 2006	22	8 / 511	.0005 - .093	.0003 - .002	100% - 100%	0.093 (fresh spinach)		
Crop group 4A - Leafy greens									
Lettuce	PDP, 2005 PDP, 2004	10	1 / 743 3 / 743	.004 .003 - .005	.0003 - .002 .001 - .002	4% - 2%	0.005	RDF5	0.001012
Spinach (fresh)	PDP, 2006	22	8 / 511	.0005 - .093	.0003 - .002	2% - 1%	0.093	RDF6	0.001
Spinach (canned)	PDP, 2004	22	0 / 371	n/a	.008	2% - 1%	0.008	RDF7	0.0042
Dandelion leaves Endive Parsley leaves	PDP, 2006	22	8 / 511	.0005 - .093	.0003 - .002	100% - 100% 100% - 5%	0.093 (fresh spinach)		
Crop group 4B - Leafy petioles									
Celery	PDP, 2007	3	6 / 739	.0005 - 4.8	.0003 - .002	4% - 1%	4.8 [f]	RDF8	0.001
Cardoon Celtuce Fennel, Florence Rhubarb Swiss chard	PDP, 2007	3	6 / 739	.0005 - 4.8	.0003 - .002	100% - 100%	4.8 (celery) [f]	4.8 (celery) [e, f]	

Crop group 5A - Brassica: head and stem									
Broccoli	PDP, 2007 PDP, 2006	10	2 / 736 0 / 185	.001 - .003 n/a	.001	6% - 3%	0.003	RDF9	0.0005
Broccoli, Chinese Brussels sprout Cabbage Cabbage, Chinese, napa Cabbage, Chinese, mustard Cauliflower Kohlrabi	PDP, 2007 PDP, 2006	10	2 / 736 0 / 185	.001 - .003 n/a	.001	100% - 100% 5% - <1% <2.5% - <1%	0.003 (broccoli)		
Crop group 5B - Brassica: leafy greens									
Collard	PDP, 2007 PDP, 2006	10	2 / 353 0 / 86	.61 - 1.7 n/a	.002 - .010 .002 / .010	11% - 5%	1.7	RDF10	0.001
Kale	PDP, 2007 PDP, 2006	10	1 / 383 0 / 98	.23 n/a	.002 - .010 .002 - .010	2% - 1%	0.23	RDF11	0.001
Broccoli raab Cabbage, Chinese, bok choy Mustard, greens Rape, greens Turnip, greens	PDP, 2007 PDP, 2006	10	2 / 353 0 / 86	.61 - 1.7 n/a	.002 - .010 .002 - .010	100% - 100%	1.7 (collard)	RDF80 (collard data for turnip greens only)	
Crop group 6 - Soybean									
Soybean	PDP, 2005 PDP, 2004	.5	0 / 668 0 / 586	n/a n/a	.003 .003	100% - 100% / blended	0.003	RDF12	0.0015

Crop group 6A - Edible-pod legume vegetables									
Bean, snap, succulent (green beans)	PDP, 2007 PDP, 2005	10	7 / 739 2 / 181	.011 - .32 .017 - .53	.002 - .010 .002 - .010	14% - 10%	0.53	RDF71	0.001909
Bean, snap, succulent, canned (green beans)	PDP, 2004	10	0 / 185	n/a	.002 - .010		0.010	RDF72	0.001
Bean, snap, succulent, frozen (green beans)	PDP, 2005	10	5 / 555	.003 - .071	.002 - .010		0.071	RDF73	0.001088
Pea, edible podded, succulent (sweet peas)	PDP, 2006 (frozen) PDP, 2003 (frozen) PDP, 2002 (can./froz.) PDP, 2001	10	1 / 722 0 / 54 0 / 122 0 / 54	.052 n/a n/a n/a	.002 - .010 .008 .008 .008	1% - 1%	0.052	RDF74	0.002543
Crop group 6B - Dried shelled peas / beans (except soybeans)									
Bean, black, seed Bean, broad, seed Bean, cowpea, seed Bean, great northern, seed Bean, kidney, seed Bean, lima, seed Bean, mung, seed Bean, navy, seed Bean, pink, seed Bean, pinto, seed Chickpea, seed Guar, seed Lentil, seed Pea, dry Pea, pigeon, seed	PDP, 2005 PDP, 2004	.5	0 / 668 0 / 586	n/a n/a	.003 .003	100% - 100%	0.003 (soybean)		

Crop group 8 - Fruiting vegetables									
Eggplant	PDP, 2006 PDP, 2005	5	12 / 740 22 / 736	.017 - .18 .017 - .14	.010	13% - 9%	0.18	RDF13	0.007439
Sweet bell pepper	PDP, 2004 PDP, 2003 PDP, 2002	5	40 / 558 54 / 741 9 / 186	.001 - .81 .002 - .65 .002 - .55	.001 .001 .001	12% - 5%	0.81	RDF14	0.025591
Tomato	PDP, 2007	5	0 / 741	n/a	.006	9% - 6%	0.006	RDF15	0.003
Tomato, paste (canned)	PDP, 2001	5	2 / 369	.018 - .043	.011	100% - 100% / blended	0.043	RDF16	0.0055
Okra	PDP, 2004 PDP, 2003 PDP, 2002	5	40 / 558 54 / 741 9 / 186	.001 - .81 .002 - .65 .002 - .55	.001 .001 .001	100% - 100%	0.81 (sweet bell pepper)		
Tomatillo	PDP, 2007	5	0 / 741	n/a	.006	100% - 100%	0.006 (tomato)		
Tomato canned and puree	PDP, 2001	5	2 / 369	.018 - .043	.011	100% - 100%	0.043 (tomato, canned)	RDF16 (canned tomato paste data)	
Crop group 9A - Curcurbits: melons									
Cantaloupe	PDP, 2005 PDP, 2004	3	20 / 558 26 / 742	.003 - .11 .003 - .61	.002 - .008 .002 - .008	13% - 9%	0.61	RDF17	0.001896
Watermelon	PDP, 2006 PDP, 2005	3	1 / 550 0 / 182	.007 n/a	.002 - .010 .002 - .010	12% - 8%	0.007	RDF18	0.001
Casaba Honeydew melon	PDP, 2005 PDP, 2004	3	20 / 558 26 / 742	.003 - .11 .003 - .61	.002 - .008 .002 - .008	100% - 100%	0.61 (cantaloupe)	RDF78 (cantaloupe data for honeydew only)	
Crop group 9B - Curcurbits: squash / cucumbers									
Cucumber	PDP, 2004 PDP, 2003 PDP, 2002	3	14 / 557 26 / 739 11 / 183	.003 - .043 .013 - .20 .013 - .063	.002 - .010 .008 .008	22% - 7%	0.20	RDF19	0.003901

Squash, summer	PDP, 2007 PDP, 2006	3	1 / 742 0 / 186	.003 n/a	.002 - .006 .002 - .006	24% - 13%	0.003	RDF29	0.001010
Squash, winter	PDP, 2006 PDP, 2005 PDP, 2004	3	0 / 369 0 / 731 0 / 364	n/a n/a n/a	.002 - .008 .002 - .008 .002 - .008	24% - 13%	0.008	RDF30	0.001
Balsam pear Chayote fruit	PDP, 2004 PDP, 2003 PDP, 2002	3	14 / 557 26 / 739 11 / 183	.003 - .043 .013 - .20 .013 - .063	.002 - .010 .008 .008	100% - 100%	0.20 (cucumber)		
Chinese waxgourd Pumpkin	PDP, 2006 PDP, 2005 PDP, 2004	3	0 / 369 0 / 731 0 / 364	n/a n/a n/a	.002 - .008 .002 - .008 .002 - .008	100% - 100%	0.008 (winter squash)		
Crop group 10 - Citrus fruits									
Grapefruit	PDP, 2006 PDP, 2005	10	3 / 743 13 / 719	.003 .003 - .039	.002 - .006 .002 - .006	11% - 6%	0.039	RDF31	0.001018
Orange	PDP, 2005 PDP, 2004	10	14 / 741 14 / 742	.003 - .12 .003 - .059	.002 - .010 .002 - .010	4% - 2%	0.12	RDF32	0.001536
Orange juice	PDP, 2006 PDP, 2005 PDP, 2004	10	165 / 557 40 / 744 21 / 186	.002 - .016 .003 - .017 .003 - .010	.001 - .010 .002 - .010 .002 - .010	100% - 100% / blended	0.017	RDF33	0.001581
Grapefruit juice	PDP, 2006 PDP, 2005	10	3 / 743 13 / 719	.003 .003 - .039	.002 - .006 .002 - .006	100% - 100%	0.039 (grapefruit)		
Citrus citron Citrus hybrid Citrus oil Kumquat Lemon Lemon juice Lime Lime juice Pummelo Tangerine Tangerine juice	PDP, 2005 PDP, 2004	10	14 / 741 14 / 742	.003 - .12 .003 - .059	.002 - .010 .002 - .010	100% - 100% <2.5% - <1% 10% - 5%	0.12 (orange)		

Crop group 11 - Pome fruits									
Apple	PDP, 2005 PDP, 2004	12	53 / 743 66 / 744	.0005 - .32 .0005 - .49	.0003 - .002 .0003 - .002	35% - 24%	0.49	RDF34	0.005804
Apple juice	PDP, 2007	12	14 / 368	.003 - .031	.002 - .020	100% - 100% / blended	0.031	RDF35	0.001937
Apple sauce	PDP, 2006	12	165 / 744	.0005 - .23	.0003 - .002	100% - 100% / blended	0.23	RDF36	0.003683
Pear	PDP, 2005 PDP, 2004	12	61 / 555 41 / 741	.003 - .33 .003 - .46	.002 - .010 .002 - .010	5% - 3%	0.46	RDF37	0.004646
Crabapple Loquat Quince	PDP, 2005 PDP, 2004	12	53 / 743 66 / 744	.0005 - .32 .0005 - .49	.0003 - .002 .0003 - .002	100% - 100%	0.49 (apple)		
Pear juice	PDP, 2005 PDP, 2004	12	61 / 555 41 / 741	.003 - .33 .003 - .46	.002 - .010 .002 - .010	5% - 1%	0.46 (pear)	RDF37 (pear)	
Crop group 12 - Stone fruits									
Cherry	PDP, 2007	10	68 / 419	.003 - .84	.002 - .004	33% - 22%	0.84	RDF38	0.026422
Nectarine	PDP, 2007	10	35 / 563	.010 - 1.5	.006	13% - 6%	1.5	RDF39	0.010707
Peach	PDP, 2007 PDP, 2006	10	87 / 555 25 / 90	.002 - .67 .002 - .45	.001 - .002 .001 - .002	13% - 6%	0.67	RDF40	0.008962
Plum (prune, fresh)	PDP, 2006 PDP, 2005	10	50 / 515 49 / 573	.017 - .56 .017 - .11	.010 .010	8% - 5%	0.56	RDF41	0.006179
Plum (dried)	PDP, 2006 PDP, 2005	10	1 / 224 7 / 153	.043 .017 - .45	.010 .010	5% - <1%	0.45	RDF42	0.005
Cherry juice	PDP, 2007	10	68 / 419	.003 - .84	.002 - .004	100% - 100%	0.84 (cherry)		
Apricot Apricot (dried)	PDP, 2007	10	35 / 563	.010 - 1.5	.006	100% - 100%	1.5 (nectarine)	RDF77 (nectarine)	
Peach juice	PDP, 2007 PDP, 2006	10	87 / 555 25 / 90	.002 - .67 .002 - .45	.001 - .002 .001 - .002	100% - 100%	0.67 (peach)		
Plum, prune, fresh Plum, prune, juice	PDP, 2006 PDP, 2005	10	50 / 515 49 / 573	.017 - .56 .017 - .11	.010 .010	100% - 100%	0.56 (plum)		

Crop group 13A - Caneberry group									
Raspberry	FDA, year not known [j]	10	51 / 247	.005 - 3.79	not known [j]	7% - 3%	3.79	RDF43	0.092575
Raspberry juice	FDA, year not known [j]	10	51 / 247	.005 - 3.79	not known [j]	7% - 3%	3.79	RDF43 (raspberry)	0.092575
Blackberry Blackberry juice Boysenberry Dewberry Loganberry	FDA, year not known [j]	10	51 / 247	.005 - 3.79	not known [j]	100% - 100%	3.79 (raspberry)	RDF79 (raspberry data for blackberry and blackberry juice only)	
Crop group 13B - Bushberry group									
Blueberry	FDA, year not known [j]	10	23 / 212	.005 - 9.7	.002	44% - 22%	9.7	RDF44	0.094618
Currant Elderberry Gooseberry Huckleberry	FDA, year not known [j]	10	23 / 212	.005 - 9.7	.002	100% -100%	9.7 (blueberry)		
Crop group 14 - Tree nuts									
Almond	PDP, 2007	0.1	0 / 361	n/a	.015	1% - 1%	0.015	RDF45	0.0075
Brazil nut Butternut Cashew Chestnut Filbert Hickory nut Macademia nut Pecan Pistachio Walnut [k]	PDP, 2007	0.1 1	0 / 361	n/a	.015	100% -100% 15% - 10% 5% - 5%	0.015 (almond)		
Crop group 15 - Cereal grains									

Sweet corn	PDP, 2003 (frozen) PDP, 2002 (can./froz.) PDP, 2001 (canned)	.02	0 / 547 0 / 727 0 / 181	n/a n/a n/a	.008 .008 .008	1% - 1%	0.008	RDF48	0.004
Corn syrup	PDP, 1998 PDP, 1999	5	0 / 298 0 / 140	n/a n/a	.006 .006	1% - 1%	0.006	RDF49	0.003
Rice	PDP, 2000 PDP, 2001 PDP, 2002	5	3 / 178 11 / 436 2 / 417	.010 - .040 .010 - .038 .010	.006 .006 .006	1% - 1%	0.040	RDF50	0.003548
Wheat	PDP, 2006 PDP, 2005	1	0 / 687 0 / 634	n/a n/a	.006 .006	1% - 1%	0.006	RDF51	0.003
Wheat flour	PDP, 2003 PDP, 2004	3	0 / 606 0 / 725	n/a n/a	.006 .006	1% - 1%	0.006	RDF52	0.003
Sorghum grain Sorghum syrup	PDP, 2003 (frozen) PDP, 2002 (can./froz.) PDP, 2001 (canned)	.02	0 / 547 0 / 727 0 / 181	n/a n/a n/a	.008 .008 .008		0.008 (sweet corn)		
Millett	PDP, 2006 PDP, 2005	1	0 / 687 0 / 634	n/a n/a	.006 .006		0.006 (wheat)		
Crop group 19A - Herbs									
Dillweed Parsley, dried leaf	PDP, 2006	22	8 / 511	.0005 - .093	.0003 - .002	100% - 100%	0.093 (fresh spinach)		
Crop group 20 - Oilseed									
Sunflower seed	Field trial, 1995 [h]	.5	8 / 20	<.02 - .129	.02	100% - 100% / blended	0.129		
Sunflower oil	Field trial, 1995 [i]	1	0 / 4	n/a	<.02 [7]	100% - 100% / blended	0.02		
Flaxseed oil	Field trial, 1995	1	0 / 4	n/a	<.02 [7]	100% - 100%	0.02 (sunflower oil)		
Crop group O - Other									

Asparagus	PDP, 2003 PDP, 2003 (canned) PDP, 2002	15	5 / 351 0 / 354 6 / 708	.013 - 2.5 n/a .020 - 15	.008 - .010 .008 - .010 .008 - .020	45% - 35%	15 - fresh 0.010 - canned	RDF53 RDF54 (canned)	0.004665 0.004609 (canned)
Banana	PDP, 2007 PDP, 2006	5	1 / 744 1 / 742	.003 .003	.002 - .010 .002 - .010	100% - 100% (imported)	0.003	RDF55	0.001012
Cactus (prickly pear fruit and pad)	no measurements	5 for fruit, 12 for pad	n/a	n/a	n/a	100% - 100%	0.025	0.025	
Cranberry	PDP, 2006	3	12 / 316	.003	.002 - .018	68% - 32%	0.003	RDF56	0.001267
Grape	PDP, 2005 PDP, 2004	10	34 / 739 35 / 738	.003 - .47 .003 - .58	.002 - .010 .002 - .010	10% - 7%	0.58	RDF57	0.00915
Grape juice	PDP, 1999 PDP, 1998	10	290 / 714 245 / 665	.003 - .086 .007 - .044	.002 - .025 .004 - .025	100% - 100% / blended	0.086	RDF58	0.004838
Raisin	PDP, 2007 PDP, 2006	10	4 / 372 9 / 372	.003 - .12 .008 - .036	.002 - .006 .002 - .006	10% - 7%	0.12	RDF59	0.001051
Olive	Field trial, 1995 [g]	10	12 / 12	.83 - 9.83	.01	2% - 1%	9.83	RDF76	
Olive oil	Field trial	USEPA (2007b) cited - details of study not available					0.077	0.077	
Peanut butter	PDP, 2006	.05	0 / 739	n/a	.010	100% - 100% / blended	0.010	RDF60	0.005
Pineapple	PDP, 2000 PDP, 2001 PDP, 2002	2	4 / 364 7 / 730 9 / 360	.017 - .043 .013 - .060 .013 - .11	.008 - .010 .008 - .010 .008 - .010	100% - 100% (import)	0.11	RDF61	0.004927
Strawberry	PDP, 2005 PDP, 2004	4	21 / 733 36 / 729	.003 - .44 .003 - 1.1	.002 .002	27% - 17%	1.1	RDF62	0.003625
Water, drinking (“finished water”)	PDP, 2007 PDP, 2006 PDP, 2005	no tol.	0 / 369 0 / 365 1 / 374	n/a n/a 19 ppt	12 - 23 ppt 12 - 23 ppt 4.7 - 23 ppt	100% - 100%	0.000019	RDF63	0.000009
Water, bottled	PDP, 2006 PDP, 2005	no tol.	0 / 367 0 / 211	n/a n/a	7.5 ppt 7.5 ppt	100% - 100%	0.0000075	RDF64	0.000004
Peanut	PDP, 2006	.05	0 / 739	n/a	.010		0.010 (peanut butter)	0.010 (peanut butter data)	

Strawberry juice	PDP, 2005 PDP, 2004	4	21 / 733 36 / 729	.003 - .44 .003 - 1.1	.002 .002	27% - 17%	1.1	RDF62 (strawberry data)	
Crop group M - Meat									
Beef, fat[18]	PDP, 2001 PDP, 2002	.5	0 / 270 0 / 301	n/a	14 ppb 14 ppb	100% - 100%	0.014	0.014	0.007
Beef, liver	PDP, 2001 PDP, 2002	3	0 / 311 0 / 313	n/a	3.6 ppb	100% - 100%	0.0036	0.0036	0.0018
Beef, muscle	PDP, 2001 PDP, 2002	1	0 / 285 0 / 310	n/a	3.6 ppb	100% - 100%	0.0036	0.0036	0.0018
Pork, fat	PDP, 2005	.5	7 / 352	.001 - .0986	.0006	100% - 100%	0.0986	0.0986	0.003
Pork, muscle	PDP, 2005	1	10 / 352	.0005 - .0066	.30	100% - 100%	0.0066	0.0066	0.000182
Goat, meat Horse, meat Sheep, meat	PDP, 2001 PDP, 2002	1	0 / 285 0 / 310	n/a	3.6 ppb	100% - 100%	0.0036 (beef muscle)		
Beef, meat by-products Beef, kidney Goat, meat by-products Goat kidney Goat liver Sheep, meat by-products Sheep kidney Sheep liver	PDP, 2001 PDP, 2002	3	0 / 311 0 / 313	n/a	3.6 ppb	100% - 100%	0.0036 (beef liver)		
Goat fat Sheep fat	PDP, 2001 PDP, 2002	.5	0 / 270 0 / 301	n/a	14 ppb 14 ppb	100% - 100%	0.014 (beef fat)		

Crop group D - Dairy products									
Milk	PDP, 2005 PDP, 2004	1	2 / 746 0 / 739	.000083 n/a	.000050 .00012	100% - 100% / blended	0.000083	RDF70	0.000043
Milk (heavy cream)	PDP, 2007 PDP, 2005	0.3	0 / 742 2 / 369	n/a .0008 - .0036	.025 .0005	100% - 100% / blended	0.0036	RDF75	0.008384

Note: this table includes only the most significant crops in the appropriate crop groups. For a complete listing of commodities included in the dietary analysis, see Appendix xxx.

[a] Percent crop treated data were retrieved from the USEPA's Acute, Probabilistic Aggregate Dietary (Food and Drinking Water) Exposure and Risk Assessments for the Reregistration Eligibility Decision (USEPA, 2007a). "Max" refers to the estimated maximum value used for the acute assessment. "Avg" refers to the weighted average value used for the chronic assessment. Both values are listed for each commodity ("max" value first, then "avg" value), as indicated by the USEPA data. If surrogate data are used, it is so indicated.

[b] Point estimates, expressed in units of ppm (µg/kg/day), represent the highest residue detection value measured from any of the latest 1-3 years of PDP testing. If insufficient data were available from these years, earlier data were accessed. In cases for which neither PDP data nor a suitable replacement crop were available, FDA or field test data were consulted. In cases in which no residues were detected, the highest Limit of Detection value was used as the point estimate. For PDP-based data, nearly all commodities were sampled in California. The exceptions to this were corn syrup, soybeans and "finished" drinking water. Surrogates were used within crop groups.

[c] Monte Carlo estimations were carried out using residue data files (RDFs) set up for each commodity for which residue distribution data were available. In cases where a surrogate was used, it is indicated in parentheses under the RDF number in this column.

[d] Chronic residue estimates represent the average of all measured residue values. In the case of non-detections, one-half of the LOD value was used. Percent crop treated data were not applied to the chronic residue calculation.

[e] When surrogates were used in the Monte Carlo analysis, percent crop treated values were set to 100%.

[f] Acute point estimates for celery and for commodities which used celery PDP data were set at the celery tolerance of 3 ppm because of the tolerance excedence measurement of 4.8 ppm. Monte Carlo and chronic estimations utilized the entire residue data set for celery.

[g] Olive field trial: Macy and Lee (1995)

[h] Sunflower seed field trial: Robinson (1995a)

[i] Sunflower oil field trial: Robinson (1995b)

[j] Residue data for various types of berries were provided by the USEPA.

[k] The Tier 2 point estimate for walnuts, which used almond data as a surrogate, presented a unique case. See text footnote #16.

Table IV-3. Acute dietary exposure to carbaryl: point estimates and Monte Carlo estimates [a]

Population subgroup	Point estimates, mg/kg/day		Monte Carlo estimates, mg/kg/day		
	95th percentile [b]	99th percentile	95th percentile [b]	99th percentile	99.9th percentile
1. US population	.006153	.015857	.000432	.001579	.006066
2. Western region	.007187	.017583	.000551	.001968	.007500
3. Hispanics	.005234	.011574	.000511	.001827	.007651
4. Non-hispanic whites	.006434	.017359	.000391	.001410	.006483
5. Non-hispanic blacks	.005274	.010680	.000447	.001544	.005429
6. Non-hispanic / non-white / non-black	.007145	.013094	.001059	.003179	.005331
7. All infants	.014589	.037382	.000661	.002532	.010644
8. Nursing infants <1 yr	.013898	.026562	.000431	.001308	.004201
9. Non-nursing infants <1 yr	.014953	.037608	..000771	..002810	.012796
10. Females 13+ (preg./not lact.) [c]	.004960	.010054	.000373	.001983	.001991
11. Females 13+ (lactating) [c]	.004357	.008433	.000334	.000639	.001439
12. Children 1-2 yr	.014194	.031084	.000905	.002291	.010476
13. Children 3-5 yr	.011654	.024534	.000799	.002066	.008455
14. Children 6-12 yr	.007621	.018623	.000524	.001393	.007275
15. Youth 13-19 yr	.004087	.011155	.000357	.001209	.004290
16. Adults 20-49 yr	.004308	.011805	.000345	.001255	.003584
17. Adults 50+ yr	.005240	.015709	.000329	.002170	.007442
18. Females 13-49 yr	.004568	.012001	.000325	.001240	.003564
19. M/F 16-70 yr	.004546	.012049	.000344	.001403	.004354

[a] These estimates were generated by the DEEM-FCID® program, which utilized consumption data from 1994-1996 USDA food consumption survey along with the 1998 Supplemental Children's Survey (CSFII 1998). Point estimates from the PDP program were the high estimates or high LODs on each commodity bearing a tolerance that was assayed in that program. Monte Carlo analysis was based on 500 iterations, a seed number of 10, and incorporated PCT data on non-blended residue distributions (PCT was set to 100% for blended commodities). All residue distribution files (RDFs) came from the PDP data base, with the exception of the artificial RDF constructed for olives, which utilized field trial data, and the RDFs for raspberries and blueberries, which originated with the FDA.

[b] Estimated percentile of user-days falling below the calculated exposure.

[c] The total number of user days for "Females 13+ (preg./not lactating)" and "Females 13+ (lactating)" were 140 and 80, respectively. These sample sizes were very small compared to the other subpopulations, for

which the total user days ranged between 583 (“Nursing infants <1 yr”) and 40,223 (“US population”). The representativeness of the two former populations was therefore subject to uncertainty.

Table IV-4. Chronic dietary exposure to carbaryl

Population subgroup	Chronic exposure estimates, PDP data mg/kg/day [a]
1. US population	0.000287
2. Western region	0.000364
3. Hispanics	0.000266
4. Non-hispanic whites	0.000291
5. Non-hispanic blacks	0.000251
6. Non-hispanic / non- white / non-black	0.000379
7. All infants	0.000503
8. Nursing infants <1 yr	0.000235
9. Non-nursing infants <1 yr	0.000604
10. Females 13+ (preg./not lact.)	0.000193
11. Females 13+ (lactating)	0.000244
12. Children 1-2 yr	0.000655
13. Children 3-5 yr	0.000483
14. Children 6-12 yr	0.000322
15. Youth 13-19 yr	0.000225
16. Adults 20-49 yr	0.000253
17. Adults 50+ yr	0.000263
18. Females 13-49 yr	0.000249
19. Males/Females 16-70 yr	(Not reported: DEEM-FCID® did not include this population in its chronic program)

[a] All non-detects were set at half of the highest LOD value measured for each commodity.

C. RISK CHARACTERIZATION

1. Acute risk estimation - deterministic and distributional approaches

Acute dietary risk estimates using the deterministic (point estimate) and distributional (Monte Carlo) exposure predictions appear in Table IV-5. This table contains MOEs derived from the largely PDP-based exposure estimates. Both critical regulatory values used to assess acute risk - the acute NOEL of 1 mg/kg and the acute LED_{10} of 0.25 mg/kg - were derived from the rat developmental neurotoxicity study of Robinson and Broxup (1997). The former value was based on clear FOB signs and statistically significant on body weight gain decrements at 10 mg/kg. The latter was based on the induction of slight hypotonic gait at 1 mg/kg. These endpoints were relevant to dietary assessment because they were established in a study that employed oral gavage as the route of exposure.

NOEL = 1 mg/kg. Tier 2 / point estimate-derived acute MOEs (Table IV-5) calculated using a NOEL of 1 mg/kg were below the health protective standard of 100 for several subpopulations at the 95th user day percentile. These included all infants (MOE=68), nursing infants <1 yr (MOE=68), non-nursing infants <1 yr (MOE=64), children 1-2 yr (MOE=68) and children 3-5 yr (MOE=84). At the 99th user day percentile, all populations except for lactating females 13+ had MOEs less than 100, with MOEs reaching as low as 24 for all infants and non-nursing infants <1 yr. A refined Tier 3 / Monte carlo analysis was triggered because of these low values.

As expected, the Monte Carlo-derived MOEs were higher than the corresponding point estimates, commensurate with their lower calculated exposures. There were no subpopulations exhibiting MOEs below 100 at the 95 or 99th user day percentiles using Monte Carlo analysis. However, at the 99.9th percentile, three subpopulations showed MOEs below 100: all infants (MOE=91), non-nursing infants <1 yr (MOE=76) and children 1-2 (MOE=92).

LED_{10} = 0.25 mg/kg. As the Tier 2 / point estimate data show (Table IV-5), all subpopulations exhibited acute MOEs less than the health protective standard of 100 at the 95th and 99th user day percentiles. The lowest MOEs at any percentile were found among infants, reaching 6 at the 99th percentile and 16 at the 95th percentile for non-nursing infants <1 yr. Furthermore, MOEs of 6 and 19 at the 99th and 95th percentiles, respectively, were determined for all infants, and 9 and 17 for nursing infants <1 yr. Because these values were so low, Tier 3 / Monte Carlo analysis was triggered.

Again, the Monte Carlo-derived MOEs were higher than the corresponding point estimates due to their lower calculated exposures. Even so, with two exceptions, all MOEs at the 99.9th percentile fell below the health-protective standard of 100 (those exceptions, females 13+ pregnant / non-nursing and females 13+ years nursing, did not provide reliable exposure estimates due to the very low number of user days available for analysis - see footnote “c”, Table IV-3). As with the point estimate data, the lowest MOEs occurred among infants and young children 1-2 years, reaching 19 for non-nursing infants <1 yr at the 99.9th percentile. Even at the 99th percentile there were many subpopulations registering MOEs below 100, including one (non-hispanic non-white non-black) that included adults. All subpopulations showed MOEs greater than 100 at the 95th percentile in the Monte Carlo analysis.

2. Chronic risk estimation

Chronic dietary risk estimates appear in Table IV-6. The chronic LED_{10} of **0.5 mg/kg/day** (ED_{10}=1.7 mg/kg/day) was used to assess chronic dietary risk. It was determined by applying benchmark dose modeling (Hill algorithm) to the brain cholinesterase activity profile in female beagle dogs after 52 weeks of dietary exposure to carbaryl (Hamada [1987]). Statistically significant inhibition was noted at all doses, including the low dose of 3.7 mg/kg/day. As the MOEs ranged between 827 (non-nursing infants <1 yr) and over 2000 (including, interestingly, nursing infants), a chronic dietary health concern was not indicated.

3. Oncogenic risk estimation

The oncogenic dietary risk estimate for the US population also appears in Table IV-6. The 95% upper bound potency estimate of **1.01×10^{-2} mg/kg/day^{-1}** (quantal linear algorithm) was used in this calculation. The resulting risk value, 2.90×10^{-6} for the US population, exceeded the target value of 10^{-6} used to distinguish negligible and non-negligible levels of oncogenic risk in adult populations. The highest risk level of 3.83×10^{-6} was calculated for the non-hispanic / non-white / non-black subpopulation. The lowest risk level of 2.54×10^{-6} was calculated for the non-hispanic black subpopulation.

Table IV-5. Acute dietary margins of exposure calculated using two regulatory endpoint values and two approaches to exposure estimation (point estimate and Monte Carlo [a])

Population subgroup	Point estimate MOEs [b]		Monte Carlo MOEs [b]		
	95th percentile [c]	99th percentile	95th percentile [c]	99th percentile	99.9th percentile
1. US population	160 / 40	60 / 15	2312 / 578	632 / 158	164 / 41
2. Western region	136 / 34	56 / 14	1812 ./ 453	508 / 127	132 / 33
3. Hispanics	188 / 47	84 / 21	1956 / 489	544 / 136	128 / 32
4. Non-hispanic whites	152 / 38	56 / 14	2552 / 638	708 / 177	152 / 38
5. Non-hispanic blacks	188 / 47	92 / 23	2236 / 559	644 / 161	184 / 46
6. Non-hispanic / non-white / non-black	136 / 34	67 / 19	940 / 235	312 / 78	184 / 46
7. All infants	68 / 17	24 / 6	1512 / 378	392 / 98	92 / 23
8. Nursing infants <1 yr	68 / 17	36 / 9	2316 / 579	764 / 191	236 / 59
9. Non-nursing infants <1 yr	64 / 16	24 / 6	1296 / 324	352 / 88	76 / 19
10. Females 13+ (preg./not lact.) [d]	200 / 50	96 / 24	2680 / 670	504 / 126	500 / 125
11. Females 13+ (lactating)[d]	228 / 57	116 / 29	2996 / 749	1564 / 391	692 / 173
12. Children 1-2 yr	68 / 17	32 / 8	1104 / 276	436 / 109	92 / 23
13. Children 3-5 yr	84 / 21	40 / 10	1252 / 313	484 / 121	116 / 29
14. Children 6-12 yr	128 / 32	52 / 13	1908 / 477	716 / 179	136 / 34
15. Youth 13-19 yr	244 / 61	88 / 22	2800 / 700	824 / 206	232 / 58
16. Adults 20-49 yr	232 / 58	84 / 21	2900 / 725	796 / 199	276 / 69
17. Adults 50+ yr	188 / 47	60 / 15	3032 / 758	460 / 115	132 / 33
18. Females 13-49 yr	216 / 54	80 / 20	3076 / 769	804 / 201	280 / 70
19. M/F 16-70 yr	216 / 54	80 / 20	2900 / 725	712 / 178	228 / 57

[a] For details regarding the inputs to the DEEM-FCID® program, see the text and footnote "a", Table IV-3.

[b] MOE (margin of exposure) = NOEL / exposure dose or LED_{10} / exposure dose. The NOEL = 1 mg/kg; the LED_{10} = 0.25 mg/kg. The first value in the top line of each box represents the MOE calculated with the 1 mg/kg NOEL, the second with the 0.25 mg/kg LED_{10}. The precise MOE may differ slightly from the quotient of the NOEL (or LED) ÷ exposure dose due to rounding. This may be accented with the 1 mg/kg MOE because it was calculated by multiplying the DEEM-derived 0.25 mg/kg MOE by 4.

[c] Estimated percentile of user-days falling below the calculated exposure.

[d] See footnote "c", Table IV-3.

Table IV-6. Chronic dietary margins of exposure (MOEs) and oncogenic risk values

Population subgroup	Chronic MOEs [a], PDP data mg/kg/day	Oncogenic risk [a]
1. US population	1741	2.90×10^{-6}
2. Western region	1373	3.68×10^{-6}
3. Hispanics	1882	2.68×10^{-6}
4. Non-hispanic whites	1719	2.94×10^{-6}
5. Non-hispanic blacks	1990	2.54×10^{-6}
6. Non-hispanic / non-white / non-black	1320	3.83×10^{-6}
7. All infants	995	not reported
8. Nursing infants <1 yr	2128	not reported
9. Non-nursing infants <1 yr	827	not reported
10. Females 13+ (preg./not lact.) [3]	2590	not reported
11. Females 13+ (lactating) [3]	2046	not reported
12. Children 1-2 yr	763	not reported
13. Children 3-5 yr	1036	not reported
14. Children 6-12 yr	1554	not reported
15. Youth 13-19 yr	2219	not reported
16. Adults 20-49 yr	1973	not reported
17. Adults 50+ yr	1903	not reported
18. Females 13-49 yr	2004	not reported
19. Males/Females 16-70 yr	Not reported: DEEM-FCID® does not include this population in its chronic program.	not reported

[a] The DEEM-FCID® program was used with the following input parameters: critical chronic LED_{10}=0.5 mg/kg/day, 95% upper bound on oncogenic potency = 1.00×10^{-3} $mg/kg/day^{-1}$

V. RISK APPRAISAL

Risk assessment is the process by which the toxicity of a compound is compared to the potential for human exposure under specific conditions, in order to estimate the possible risk to human health. Every risk assessment has inherent limitations relating to the relevance and quality of the available toxicity and exposure data. Assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment and exposure-assessment processes. This results in uncertainty in the risk characterization, which integrates the information from the preceding three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the magnitude of the uncertainty varies with the availability and quality of toxicity and exposure data, and the relevance of that data to the anticipated exposure scenarios;

In the following sections, the specific areas of uncertainty associated with the characterization of health risks from dietary exposure of both workers and the general public to carbaryl and its primary metabolite are described.

A. HAZARD IDENTIFICATION

Selection of the appropriate laboratory animal toxicity studies to characterize human risk is a central task of pesticide risk assessment. Two factors influence the selection process: (1) the scientific quality of the studies in question, including the reliability of the data used to support the selection of critical LOELs, NOELs and LEDs, and (2) the relevance of the routes of exposure employed in those studies to the anticipated routes of human exposure in the field. These factors are discussed in the following sections as they relate to acute and annual (chronic) exposure to carbaryl.

1. Non-oncogenic effects

a. Acute oral toxicity

NOEL = 1 mg/kg. Acutely induced FOB signs - slight tremors, slight hypotonic gait, slight ataxic gait and pinpoint pupils - combined with body weight gain decrements in pregnant rats at a gavage dose of 10 mg/kg (Robinson and Broxup, 1997) formed the basis for the critical acute NOEL determination for carbaryl. The strength of this determination lay partly in the clarity of the incidence data - their statistical significance and unarguably acute nature - and partly in the support forthcoming from three additional acute oral gavage studies from the same laboratory. Each of those studies established cholinergic LOELs at 10 mg/kg/day, but did not establish NOELs (Brooks and Broxup, 1995b and 1995c; Brooks *et al*., 1995). Further support came from the rat acute gavage study by Moser (2007) which established LED_{10}s (ED_{10}) of 1.1 (1.5) mg/kg based on brain cholinesterase inhibition and 0.78 (1.11) mg/kg based on RBC cholinesterase inhibition in postnatal day 11 animals, and from the acute inhalation toxicity study of Weinberg (2008), which established an LED_{10} (ED_{10}) based on brain cholinesterase inhibition that was equivalent to an internal dose of 0.66 (1.59) mg/kg, about halfway between the critical acute NOEL of 1 mg/kg and the LED_{10} of 0.25 mg/kg..

The major uncertainty lay in the distinct possibility that cholinergic signs - particularly slight hypotonic gait, but possibly also slight tremors - were also present at 1 mg/kg, making that dose a LOEL rather than a NOEL. The uncertainties surrounding a LOEL determination at 1 mg/kg are discussed in the following paragraphs.

$\underline{LED_{10} = 0.25 \text{ mg/kg}}$. Acutely induced slight hypotonic gait occurring in the course of FOB testing in pregnant rats at 1 mg/kg (Robinson and Broxup, 1997) was the basis for the critical acute oral LED_{10} determination of 0.25 mg/kg for carbaryl. There were several uncertainties associated with this determination:

1. In six FOB tests conducted during gestation and five conducted within 21 days of the end of gestation, statistical significance with respect to controls was achieved only once at 1 mg/kg (gd 12; $p<0.05$) and once at 10 mg/kg (gd 18; $p<0.01$). In fact, the statistical significance observed at 1 mg/kg on gd 12 was not supported by an equivalent statistically significant response at 10 mg/kg on the same day. The low level of statistical verification of the effect accentuated the possibility that slight hypotonic gait was not a response to carbaryl exposure, at least at 1 mg/kg. However, examination of the dose-response curve in Figure 1 conflicts with this scenario, as does the dose responsiveness evident when the data were averaged over the entire gestation period (Table IV-1, which is represented, in effect, by the line in Figure 1).

2. An effect of dosing on slight hypotonic gait may not have appeared until gd 9 (*i.e.*, after four applications) or gd 12, when a statistically significant increase was noted at 1 mg/kg. No effect was discernable at 1 mg/kg on gd 6. Thus the timing of the slight hypotonic gait effect might not be consistent with a classically acute response, if defined as occurring as a result of a single dose. However, as explained above in the Hazard Identification section (IV.A.1.a.), carbaryl's propensity for clearance from the rat system in less than 24 hours (Struble, 1994) combined with its relatively fast decoupling from the cholinesterase enzyme were considered strong evidence that each FOB test comprised an independent acute assay of carbaryl's neurotoxic effects.

3. Most of the FOB parameters appearing in Table III-15a were classified by the investigators as "slight" responses ("slight hypotonic gait", "slight ataxic gait", "slight tremors"). This emphasized the subjectivity of the data, since a judgment of "slight" in the hands of one observer either may not have sufficed for a notification or been classified as moderate in the eyes of another evaluator.

4. The most scientifically credible route toward establishing an acute regulatory value using the incidence data for slight hypotonic gait was to model those data using the BMD approach. This avoided the pitfalls associated with setting LOEL and NOEL values, allowing more of the data set to be used to determine the critical value. However, there were uncertainties inherent in the BMD approach. First, there was uncertainty associated with chosen benchmark response level of 10%, since it was not known if slight hypotonic gait comprised a centrally or peripherally-based response. If centrally-based, for example, the risk represented by slight hypotonic gait might be better characterized by a benchmark response level of 5% rather than 10%, which is associated with milder effects. Second, the decision to delete the top dose, which was made in order to generate a curve of appropriate fit and an LED_{10} value of lower than 1 mg/kg (a dose that almost certainly generated effects), added uncertainty since it ignored actual data gathered in the experiment. Third, the choice of the probit function over other algorithms added uncertainty because each algorithm generated different LED_{10} and ED_{10} values. And fourth, the decision to model the data using normalized mean incidence rates, which was a consequence of considering all of the FOB tests to be acute in nature, added uncertainty because it implied that data from single test days

represented fluctuations around a mean. While this was considered the more likely scenario, it remained possible that it underestimated the sensitivity of the system.

Uncertainty was also introduced into the critical study and the support studies with the gavage approach, as food intake over a single "eating occasion" was likely to result in more gradual pesticide exposure than would occur after gavage. Depending on the pharmacokinetics of carbaryl toxicity, in particular whether acute toxicity is most influenced by the highest achieved concentration or the total concentration over a finite time span (*i.e.*, the area under the time-*vs.*-concentration curve), gavage dosing may generate a more severe response than acute dietary exposure. Also, decarbamylation of impacted cholinesterases may be more prominent under dietary, as opposed to gavage, exposure scenarios, due to the more gradual pesticide-enzyme interaction. Reactivation of cholinesterases over the exposure period would act to lessen the dietary response.

b. Chronic oral toxicity

The critical chronic oral LED_{10} of 0.5 mg/kg/day was based on inhibition of brain ChE activity after 1 year of exposure in dogs at a low dose of 3.4 mg/kg/day (actually, 3.7 mg/kg/day in females and 3.4 mg/kg/day in males; Hamada [1987]). The absence of both clinical signs and histopathology throughout the study, even at the high dose of 34 mg/kg/day, leaves open the possibility that the LED_{10} value was too low, since the enzyme inhibition was independent of overt toxicity. This uncertainty was offset by two considerations, however. First, the absence of *overt* toxicity does not necessarily signal a complete absence of toxicity, as subtle effects on learning or memory were not addressed. And second, brain ChE suppression is considered by DPR to be an adverse effect in and of itself because it is mechanistically tied to cholinergic toxicity (DPR [2002b]).

It is noted that the critical chronic oral LED_{10} (0.5 mg/kg/day) was higher than the critical acute oral LED_{10} (0.25 mg/kg), which may be considered unusual. However, this situation is not likely to compromise human health; protection against untoward acute effects at lower dose levels would ensure protection against chronic effects, regardless of their nature, resulting from exposure at higher doses.

c. Reproductive and developmental toxicity

Epidemiologic and laboratory animal data suggest that carbaryl may have adverse reproductive and/or developmental impacts. The following points may be made concerning the epidemiologic studies:

1. The relative risk for miscarriage approximately doubled in a cohort of agricultural workers when carbaryl usage by males was combined with one of two other exposure categories, including “crop herbicide application” and “application of crop insecticides and fungicides” (Savitz *et al.* [1997]).

2. Wyrobek *et al.* (1981) failed to establish “a definitive link between carbaryl exposure and human seminal defects” among workers and ex-workers in a carbaryl production facility. However, their data were suggestive of an increase in oligospermia (sperm count $<20x10^6$/ml) and teratospermia (>60% abnormal sperm forms) in that population.

3. Sperm toxicity among workers in a carbaryl production facility was evident in a recent study from China (Xia *et al.* [2005]). This was noted through the increased morphologic abnormalities, disomic and nullisomic sperm and percentages of sperm with fragmented DNA,

4. Meeker *et al* (2004a and 2004b) demonstrated a positive correspondence between urinary levels of 1-naphthol, a primary carbaryl metabolite, and various indicators of sperm toxicity among males seeking diagnoses in an infertility clinic.

The epidemiologic studies did not, however, make unambiguous associations between effects and specific xenobiotic exposures. The extent of carbaryl exposure, both with regard to time span and dose, was ill-defined and did not exclude the possibility of exposure to other risk factors. Where carbaryl exposure was suggested by the presence of a urinary metabolite, the possibility remained that the metabolite was generated from another xenobiotic - thus the studies of Meeker *et al.* (2004a and 2004b) did not unambiguously attribute the presence of 1-naphthol in the urine of subfertile males to carbaryl exposure, especially as that metabolite can also be generated from exposure to naphthalene. The study of Wyrobek *et al.* (1981), which suggested that oligospermia may be increased in carbaryl factory workers, was carried out using low subject numbers. Possibly as a consequence, the measured effects showed low statistical confidence (though it might be argued that low statistical confidence in a small study should tilt the interpretation toward a positive association). However, a more recent examination of carbaryl factory workers from China also provided evidence for sperm toxicity (Xia *et al.* [2005]). In reporting the increase in relative risk for miscarriage in wives of husbands working with carbaryl in agricultural settings, Savitz *et al.* (1997) also could not exclude a role for other chemical and environmental stressors. Taken as a whole, the epidemiologic studies suggested potential reproductive problems in exposed males, though the data were considered supportive as opposed to conclusive.

Reproductive and developmental toxicity concerns were also raised in laboratory animal studies, as noted here:

1. In the most recent and most complete gavage studies to date, Pant *et al.* (1995, 1996) demonstrated impacts on testicular enzymes, sperm counts, sperm motility, sperm morphology and testicular morphology in rats at a daily carbaryl dose of 50 mg/kg/day (5 days/week, 90 days).

2. Chronic administration of carbaryl to rats suggested adverse impacts on sperm motility, seminiferous tubule morphology, estrus cycle lengths, gonadotropic hormone levels and corpora lutea / atretic follicle numbers at doses as low as 7 mg/kg/day (Shtenberg and Rybakova [1968]).

3. Collins *et al.* (1971) demonstrated impairments in several reproductive indices in gerbils, including fertility, pups per litter, liveborn pups per litter, pup survival to days 4 and 21, and weanling weights at doses as low as ~160 mg/kg/day.

4. Smalley *et al.* (1968) demonstrated severe dystocia and other reproductive effects in pregnant beagle dogs after dietary exposure of the mothers to carbaryl at doses as low as 3.125 mg/kg/day and developmental

effects in their offspring at 6.25 mg/kg/day. In addition, Immings *et al.* (1969), also working with pregnant beagles, showed an increase in stillbirths at as low as 2 mg/kg/day and an increase in *post partum* pup deaths at 5 mg/kg/day. These studies are discussed below.

5. In a small gavage study in rats, Kitagawa *et al.* (1977) provided histological evidence for decreases in spermatogonia and spermatozoa numbers in the seminiferous tubules during a 1-year gavage study at an approximate dose of 15 mg/kg/week.

As was the case for the epidemiologic studies, there were caveats in regards to the laboratory animal studies. In particular, the means of oral exposure (gavage bolus *vs.* dietary) may have a bearing, at least in the rat, where bolus dosing was often used. Bolus exposures result in more immediate and higher blood levels of a test article than dietary exposure. A guideline rat dietary reproductive toxicity study did not reveal carbaryl-induced effects on F_0 or F_1 reproductive indices, parental epididymal sperm counts, sperm motility / morphology, homogenization-resistant spermatid head counts, daily sperm production or efficiency of daily sperm production (Tyl *et al.,* 2001). There were, however, deleterious effects on pup body weights and survival, as well as delays in developmental parameters. A dietary study in gerbils showed impairments in several reproductive indices, including fertility, pups per litter, liveborn pups per litter, pup survival to days 4 and 21, and weanling weights (Collins *et al.,* 1971), though the relevance of the gerbil system in a risk assessment framework was not clear since this species is rarely examined. Several open literature gavage studies (Rybokova, 1966; Shtenberg and Rybakova, 1968; Kitagawa *et al.,* 1977; Pant *et al.,* 1995, 1996) suggested histotoxicity in the male reproductive system of the rat, though standard reproductive indices were not measured. In a rat gavage study, Dikshith *et al.* (1976) observed no significant effects on the rate of pregnancy, litter size, number of offspring born, or on pup health and viability through 10 days after exposed males were mated with unexposed females, though carbaryl-induced changes in two testicular enzymes, succinic dehydrogenase and adenosine triphosphatase, were identified. Osterloh *et al.* (1983) did not observe effects on testicular parameters after intraperitoneal injections of male mice were carried out over a 5-day period. It was not clear if the negative result in this case was due to species insensitivity, an ineffective exposure route or other unknown factors.

As noted above, two older dog studies (Smalley *et al.,* 1968; Immings *et al.,* 1969) showed reproductive and developmental toxicity at dose levels similar to those employed in the critical acute oral study of Robinson and Broxup (1997). Protection provided by the critical acute NOEL may extend to these effects, though it is noted that the Immings study did not establish a NOEL for stillbirths. However, there were caveats in the dog studies regarding the clarity of the dose response and the applicability of the dog data to humans, which are delineated below:

(1) While both studies showed toxic effects of carbaryl in pregnant beagles and their offspring, neither produced dose response relations sufficiently convincing to set regulatory levels. For a more complete discussion of the dose response relations and other issues arising in the dog reproductive studies, see section IV.A.1.c. above.

(2) Knaak and coworkers (Knaak *et al.*, 1965, 1968; Knaak and Sullivan, 1967) concluded that carbaryl metabolism in dogs differs from that in rats and humans. Knaak considered that dogs, unlike the latter two species, do not liberate 1-

naphthol for glucuronidation or sulfation. Dogs may also be unable to hydroxylate carbaryl. If true, such characteristics could make it difficult for dogs to mount adequate detoxification reactions, making them more sensitive to carbaryl-induced toxicity (though Knaak concluded that dogs conjugate the molecule "directly" and excrete it relatively efficiently [Knaak and Sullivan, 1967]). Dogs also appear to excrete a higher portion of the carbaryl dose in the fecal fraction than rats.

If Knaak is correct, a *dog-specific* carbaryl risk would require that the unexcreted ligand was either toxic (as might obtain if more unmetabolized carbaryl or more bioactive metabolites were available) or that the dog is inherently more sensitive to carbaryl and its derivatives. Neither of these possibilities has been demonstrated.

(3) The length of exposure required to elicit the reproductive and developmental effects in dogs was unclear since exposures occured over the entire 2-month dog gestation period in the Smalley study and continued through the pre-weaning period in the Immings study. As such, it was difficult to determine if the effects were acute or subchronic in nature.

(4) Khera (1976) noted that, unlike other mammals, the dog sheds immature diploid ova, which then undergo a period of maturation and reduction to haploidy before being receptive to sperm. This could generate an altered reproductive sensitivity to xenobiotics in the dog, decreasing its relevancy as a model for potential effects in the human. However, there is no evidence at this time to support such a claim regarding carbaryl's toxicity to dogs. Indeed, the dog system provided the critical chronic oral value (see section b. below), which was certainly independent of purported effects on the chromosome content of gametes.

(5) As open literature studies, there was no empirical assurance that the carbaryl used for dosing did not contain impurities. However, this must be viewed as hypothetical, as the presence or absence of impurities in those studies was not reported. If indeed such toxicologically significant impurities exist, their properties are not known even 40+ years after the dog studies were carried out.

Even considering the possible caveats to the use of dog studies, data from rats, rabbits and mice showed some developmental impact, though probably not without accompanying maternal toxicity. In a guideline-compliant rat study, Repetto-Larsay (1998) noted an increase in the occurrence of fetal runts - defined as those with body weight $\leq$75% of control means - accompanied by delayed or absent ossification in newborns following maternal gavage at 30 mg/kg/day. These were probably related to the suppressed maternal weight gains noted at that dose. A guideline rabbit study also demonstrated a tendency toward low birth weights at the high dose of 150 mg/kg/day (Tyl *et al.* [1999]). Murray *et al.* (1979) noted a single incidence of omphalocele in a newborn rabbit after gestational exposure of the mothers to 150 mg/kg/day carbaryl by gavage. In view of the extremely low historical control rate from this laboratory (2 cases from 338 litters) and the fact that incidence rose to six newborns spread over 4 litters at 200 mg/kg/day, it was concluded that omphalocele was likely to be carbaryl induced. Bodyweight loss and diarrhea were notably present in mothers bearing offspring with

omphalocele. In the same report, dietary exposure of pregnant mice to 1166 mg/kg/day led to reduced maternal weight gains and fetal growth and ossification delays.

d. Genotoxicity

As noted above in section IV.A.1.f., carbaryl showed positive responses in one of five gene mutation studies, four of six chromosomal aberration studies and two of four DNA damage studies reviewed. However, all of the relevant positive studies were performed *in vitro*. The carbaryl metabolites nitrosocarbaryl and ∝-naphthol may also be genotoxic, though again, this has been indicated only in *in vitro* studies.

The positive genotoxicity tests may have significance in the context of a carbaryl risk assessment in view of the clear oncogenicity and possible reproductive and developmental toxicity of this compound.

2. Oncogenicity

Comparison of the dose ranges for several carbaryl-induced tumors in mice and rats suggested that the induction of hemangiosarcomas plus hemangiomas (H+H) in male mice was the most appropriate endpoint for oncogenic risk analysis. This was based on the tendency of the mouse system to develop this tumor at relatively low carbaryl doses. Since it was clear that H+H increased in several organ systems, it was assumed that development of these vascular tumors in all organ systems reflected the same biological process (McConnell *et al.* [1986]). This assumption, which allowed use of the combined organ system data for benchmark dose analysis, had uncertainties, particularly as it was not known if carbaryl's access to, or metabolic handling by, each organ was strictly equivalent. In this regard, examination of H+H incidence in the male liver -0/66, 4/66, 5/69* and 7/68**; (*,**; $p<0.05$, 0.01, respectively) - supports the plausibility of a low dose effect and suggests that the liver could indeed have special sensitivity. In fact, a dose-response relation for H+H was not apparent in any other organ in which these tumors appeared. Furthermore, if the incidence rate for total number of H+H neoplasms rather than the per-animal incidence rate is examined - 2/66, 9/66*, 13/69** and 18/68*** (*,**,***; $p<0.05$, 0.01, 0.0001, respectively) - low dose responsiveness is quite evident.

Low dose linearity was assumed in the H+H data modeling. The ability of carbaryl to induce H+H was extrapolated to zero dose after deleting the top dose, as discussed in section IV.A.2 above. An assumption of linearity may be valid in cases in which genetic damage plays a causative role. In the present case, carbaryl was clastogenic in four of six structural chromosome aberration tests and two of four DNA damage studies (though as noted in the previous section, the relevant positive studies were conducted *in vitro).* However, there was no direct evidence that carbaryl-induced H+H was caused by genotoxicity. Furthermore, a 6-month study in p53 knockout mice suggested that carbaryl may not act through a p53-based process (Chuzel [1999]). Linearized kinetics were thus invoked without an assurance that they represented an actual oncogenic process.

Omission of the high dose compromised one of the major attractions of the benchmark dose approach, to wit, its ability to take into account *all* of the doses in the analysis. In the current case it might be argued that, with few animals analyzed and fewer still responding, high dose omission is questionable, especially as a small incidence "error" in any direction at the lower doses can severely alter the slope of the incidence curve (*i.e.*, the calculated oncogenic potency). Of greater concern, however, was the striking possibility that inclusion of the high dose would result in underestimation of the oncogenicity of this compound, since it lowers the

slope of the incidence curve by a factor of nearly 10. In view of the clear evidence that the high dose exceeded the MTD, its inclusion in the potency calculation was considered to be unwise.

The assumption of low-dose linearity can be avoided by invoking a threshold and calculating a resultant MOE. Waddell (2006) provided strong evidence that threshold mechanisms are operative for most carcinogens examined in the NTP database. If a NOEL of 1.5 mg/kg/day is estimated by dividing the oncogenic LOEL of 14.73 mg/kg/day (the lowest dose tested) by an uncertainty factor of 10, the oncogenic MOE for the most highly exposed population is 3958. While this might be regarded as a low risk because it is higher that 100, it should be noted that oncogenic risk has rarely (if ever) been assessed in this way; consequently, a standard of negligible risk has not been established. USEPA's guidance document on cancer risk assessment (USEPA, 2005b) states that "a no-observed-adverse-effect level (NOAEL) generally is not used for assessing carcinogenic response when one or more models can be fitted to the data", which is the case here. In any case, the present data do not provide direct evidence for a threshold mechanism, leaving such calculations in the speculative realm.

There are major uncertainties inherent in extrapolating tumor data from rodents to humans. One facet of the species extrapolation problem that is particularly relevant to the current case concerns the relationship between spontaneous incidence and chemical inducibility for particular tumors. Does a high spontaneous incidence rate translate to a high level of chemical inducibility? And *vice versa*, would a low spontaneous rate be associated with a low level of inducibility? It is recognized, for example, that Strain A mice, an effective experimental system for the induction of lung tumors by cigarette smoke, will form lung tumors spontaneously with age (*cf.* Rubin, 2001). In the case of hemangiosarcomas, Pegg and Short (2006) pointed to much higher spontaneous incidence rates among rats and mice than among humans, raising the question of whether humans would also manifest a lesser response to a hemangiosarcoma-inducing chemical like carbaryl. The human hemangiosarcoma incidence rate as reported in the National Cancer Institute SEER database, was 0.21 new cases per 100,000 people (0.00021%) between 1996 and 2000, with the tumors occurring most commonly in skin structures from the head and neck. By contrast, the spontaneous incidence rate for B6C3F1 mice in the National Toxicology Program database was 5.4% in males and 2.7% in females (range: 0-12%). The range in Wistar rats was 0-3.4%. In addition to skin, spontaneous rodent hemangiosarcomas are commonly detected in liver, spleen, bone marrow and lymph nodes. The tendency of mice (particularly male mice) to form hemangiosarcomas, both spontaneously and through chemical induction, was also illustrated in a two-year study of mice exposed to metam sodium in drinking water (Horner, 1994; DPR, 2005). While there are no specific data to support a contention that humans are intrinsically less sensitive to hemangiosarcoma-inducing chemicals, the possibility should at least be acknowledged.

Finally, uncertainties in the oncogenic, reproductive and developmental risk analyses arise from our lack of knowledge of the effects of carbaryl degradates on these processes. This is particularly relevant in view of the fact that residues of the carbaryl degradate 1-naphthol have been detected in some food commodity samples.

B. DIETARY EXPOSURE ASSESSMENT

Uncertainties in the dietary exposure assessment fall into three major categories: (1) parameter uncertainty, (2) model uncertainty, and (3) scenario uncertainty (Peterson *et al.*, 2001).

1. Parameter uncertainty

Sources of parameter uncertainty in the dietary exposure assessment included the degree of completeness of the food residue database, the representativeness of that database to real-world exposures, the use of surrogate data, and the possible presence of sampling or reporting errors. Carbaryl residue estimates were based largely on PDP data, with resort to FDA and field trial data on particular commodities when PDP data were unavailable. In general, PDP is the database of choice for risk assessment because it is statistically designed for that purpose. Certainly with regard to the refinements represented by the Monte Carlo approach (including the use of residue distributions, percent crop treated data and processing data), PDP data are desirable, as they represent a statistically randomized approach, with samples "chosen close to the time and point of consumption (*i.e.*, distribution centers rather than farmgate)" (PDP, 2004). Furthermore, PDP aims its residue determinations at foods likely to be consumed by infants and children, which is advantageous because these are subpopulations particularly susceptible to effects induced by dietary exposure.

Several commodities - including carrots, canned spinach, soybeans, beans (snap/succulent/canned), tomatoes, winter squash, sweet corn, corn syrup, wheat, wheat flour, sunflower oil (field trial), peanut butter, bottled watter, beef fat, beef liver and beef muscle - produced no detectable residues. This meant that, for the point estimate determination, LOD values were used, thus adding uncertainty because the *actual* residue levels for these commodities could be anywhere between zero and the LOD. The distributional (Monte Carlo) approach was less problematic in this regard because non-blended commodity estimates were based on an assumption that only a fraction of the non-detects were at the LOD, depending on the percentage of the crop that was treated (PCT) (see discussion below).

Residue data from field trials were employed in the current assessment for olives, sunflower oil and sunflower seeds. Such studies were conducted to determine the highest residue level that can result from maximum legal use of the pesticide. Field trial applications did not reflect actual use patterns; the resultant residue data were used only because other data sources were unavailable. As such, they almost certainly represented overestimates of exposure from those commodities. Fortunately, none of these commodities emerged as major contributors in either the point estimate or distributional analyses, having little influence on the reliability of the overall risk estimates.

Surrogate crops were assigned when residue data from PDP, FDA or field trials were not available for commodities with tolerances. The commodity / surrogate pairs used in this assessment were derived from the crop group categorizations in 40CFR; they are listed in section IV.B.5.d. and in Table IV-2. The use of surrogates added obvious uncertainties to the analysis.

Uncertainties in the dietary exposure assessment also arose from the consumption data contained in the 1994-1998 CSFII survey. There are several possible sources for this type of uncertainty, including the representativeness of actual dietary consumption, reporting errors, or variation in dietary habits during or after the consumption period. This was particularly evident

for two subpopulations: females 13+/pregnant/not nursing and females 13+/nursing. There were so few user days represented for these groups (140 and 80, respectively) that the resultant exposure and risk estimates were considered to be unreliable.

2. Model uncertainty

The 95th and 99th user day percentiles were presented in this assessment as estimated high-end exposures when the single highest detected residue or LOD was used for commodities with tolerances (*i.e.*, in the Tier 2 / determinstc estimate). Acute dietary exposures to carbaryl, calculated at those exposure levels using the Tier 2 estimates indicated a health concern even at the 95th percentile, as MOEs for all subpopulations were below the health protective standard of 100. However, these values represent, at best, high upper bound estimates of dietary risk.

Because the point estimate data suggested the presence of risk when Tier 2 assumptions were made, a more refined distributional modeling approach (Tier 3 / Monte Carlo) was initiated. For this assessment, the 95th, 99th and 99.9th percentiles were used for this analysis. Residue distribution and PCT information were incorporated for all commodities for which distributional data were available, resulting in the creation of a series of residue distribution files using PDP-based data. With a few exceptions made for commodities lacking residue data that were surrogated because preliminary analysis indicated a probability of high contribution to the exposure profile, the remaining commodities retained their point estimate values. By virtue of their lower consumption, they did not make a substantial contribution to the total carbaryl consumption.

Tier 3 analysis generated MOEs less than 100 for three subpopulations at the 99.9th user day percentile using 1 mg/kg as the critical toxicology value and for all subpopulations using 0.25 mg/kg as the critical toxicology value. In the latter case, the only exception was females 13+/nursing, for which the data were unreliable (see above). Several subpopulations, including non-hispanic black, non-hispaninc / non-white / non-black, all infants, non-nursing infants, children 1-2 yr and adults 50+ yr, registered sub-100 MOEs using the 0.25 mg/kg critical toxicology value even at the 99th percentile. All MOEs exceeded the health-protective target of 100 at the 95th percentile using 0.25 mg/kg and at the 95th and 99th percentiles using 1 mg/kg. These calculations were, however, subject to a major uncertainty related to incompleteness of the residue data base. Despite the high number of RDFs (~70) - which functioned to lower the overall exposure estimates because they resulted in the great majority of residues within a data set to be set to zero or to the LOD (depending on the PCT) - residue levels for many highly consumed commodities were maintained at the point estimate levels assigned to them in the Tier 2 analysis due to the absence of specific data. This resulted in a certain overestimate of exposure, since most of the the 350 commodities included in the analysis were in this category. A rough sense of the extent of this overestimation is gained by assigning RDFs to all commodities within a crop group, making virtually every commodity with a reasonable surrogate subject to a residue distribution. Using this approach with the 0.25 mg/kg LED_{10}, MOEs at the 99.9th percentile were 59 for all infants (*i.e.*, ~4-fold greater than the MOE of 14 reported in Table IV-5), 57 for nursing infants <1 yr (~2-fold greater), 59 for non-nursing infants <1 yr (~4-fold greater), 59 for children 1-2 yr (~4-fold greater) and 64 for children 3-5 yr (~3-fold greater). All other subpopulations showed 99.9th percentile MOEs greater than 100. For example, the MOE for the US population was 146, ~4-fold greater than the corresponding MOE in Table IV-5. All 99th and 95th percentile MOEs were well over 100.

This exercise suggests that the exposure estimates at the 99.9th percentile could be as much as 4-5-fold less than those reported here (with consequent MOEs 4-5-fold greater). Of course the exercise imposed its own uncertainties, since RDFs, including their attendant percent crop treated values, were applied as surrogates to commodities for which there were no data. However, it also emphasizes the seriousness of the risk imposed by dietary carbaryl, particularly to infants and young children, as these subpopulations continued to register low MOEs even when surrogate distributions were applied to virtually all commodities lacking specific data.

A similar uncertainty pertained to the chronic analysis, as surrogate high acute point estimates were applied to commodities lacking specific data. This resulted in certain overestimation of exposure and understimation of MOEs, since chronic residue values were the average of residue values determined in many samples (including a majority of non-detects, which were set at one-half of the LOD value). However, as no subpopulation registered a chronic MOE lower than 869 (non-nursing infants), this point serves only to further discount the potential for risk stemming from chronic exposure to carbaryl.

Finally, it is worth noting that the distributional files included several blended commodities. As such, the “distributions” for those commodities did not include any samples that were set to zero, since blending was considered to represent a mixture of treated and untreated commodities. Nonetheless, this probably resulted in overestimates of acute exposure, since potential "zero" or sub-LOD values were removed from the analysis.

C. RISK CHARACTERIZATION

1. Non-oncogenic risk

Non-oncogenic risk was evaluated by use of the margin of exposure ratio, equivalent to the critical NOEL or LED divided by the anticipated exposure. A MOE of 100 or more is considered sufficiently protective of human health when the NOEL of LED for an adverse effect is derived from an animal study, as was the case in this assessment. The MOE of 100 assumes that humans are 10 times more sensitive than the most sensitive laboratory animals and that there is a 10-fold variation in sensitivity within the human population. Uncertainties were introduced into the MOE calculations by uncertainties in both the LED and exposure terms, and were recounted in detail in the preceding paragraphs.

The MOEs for acute dietary exposure were calculated using either of two critical toxicologic values: (1) a critical NOEL of 1 mg/kg based on cholinergic signs detected with FOB testing and decrements in body weight gain in pregnant rats at 10 mg/kg (Robinson and Broxup, 1997); or (2) a critical acute LED_{10} of 0.25 mg/kg, based on the induction of slight hypotonic gait at the LOEL dose of 1 mg/kg in pregnant rats in the same study (Robinson and Broxup, 1997). The residue exposure data were derived predominantly from the PDP database.

Tier 2 analysis using point estimates established at the highest residue level measured or, in the case of no detections, at the high LOD, generated MOEs lower than 100 at both the 95^{th} and 99^{th} user day percentiles with either critical toxicology value, triggering distributional (Tier 3) analyses in both cases. MOEs from Tier 3 analysis were above the health protective standard of 100 at the 95^{th} and 99^{th} user day percentiles for all of the subpopulations analyzed when a NOEL of 1 mg/kg was used. However, three subpopulations fell under 100 at the 99.9^{th} percentile, indicating a potential acute risk. When 0.25 mg/kg was used, MOEs were lower than 100 for several subpopulations at the 99^{th} percentile and nearly all subpopulations at the 99.9^{th} percentile. To the extent that these values were based on an LED_{10} extrapolated from data in a laboratory animal study (see discussion in section V.A.1.a. in particular), they should be considered uncertain. The exposure terms were refined by Tier 3 distributional analyses, which attempted to model the residue database in as "realistic" a manner as the data allowed through the Monte Carlo approach. However, the absence of residue data on many commodities may have resulted in as much as a 4-5-fold overestimation of total exposure, since these values were set at the highest residue value measured (see discussion above, section V.B.2.). Even with this consideration, Monte Carlo analysis generated MOEs less than 100 for several subpopulations at the 99.9^{th} percentile.

The MOEs for chronic dietary exposure were calculated using (1) the critical chronic LED_{10} of 0.5 mg/kg/day, based on inhibition of brain cholinesterase activity at 3.4 mg/kg/day and above in dogs at 52 weeks (Hamada, 1987), and (2) mean exposure data derived mostly from the PDP database. As these MOEs were uniformly high even without the incorporation of percent crop treated data (lowest MOE = 763), it was considered unlikely that chronic dietary exposure presented an appreciable chronic health risk.

2. Oncogenic risk

Oncogenic risk is expressed as the product of the projected exposure multiplied by the 95% upper bound on potency. The resultant unitless value represents the total extra cases expected as a result of "lifetime" exposure to carbaryl. In the context of the DEEM-FCID®-based dietary assessment, the amortized lifetime exposure is essentially the same as the chronic exposure output for the various adult subpopulations, which is why the range of oncogenic risk values, 2.54×10^{-6} and 3.83×10^{-6}, was calculated based on the average exposure indicated for those groups. Because they were more than the negligible risk standart 10^{-6}, dietary consumption of carbaryl was considered to constitute an oncogenic risk.

D. CRITICAL TOXICITY ENDPOINTS AND RISK CALCULATIONS - USEPA *vs.* DPR

USEPA outlined their endpoints for carbaryl in a Reregistration Eligibility Decision document (RED) dated September 2007 (USEPA, 2007a) and in a more specific chapter on toxicity dated June 2007 (USEPA, 2007b). Their conclusions are summarized and compared to the values established in the present document in the following paragraphs and in Table V-1.

Acute oral toxicity. USEPA's acute "point of departure" (PoD) was 1.1 mg/kg, an LED_{10} value derived from brain cholinesterase inhibition data in postnatal day 11 rats (Moser, 2007). The USEPA PoD was essentially equivalent to DPR's critical acute NOEL of 1 mg/kg, but ~4-fold greater than DPR's critical acute LED_{10} of 0.25 mg/kg. Combined with DPR's more selective use of surrogate residue data files in the Tier 3 / Monte Carlo exposure analysis (see section V.B.2. above), these critical values largely account for the differences between the two agencies in their estimation of risk. (DPR also used, wherever possible, only residue data collected in California.) USEPA concluded that dietary exposure to carbaryl was lower than their target value of 100% of the "acute population adjusted dose" (aPAD) at the 99.9^{th} user day percentile for all subpopulations analyzed. For example, the aPAD was 29% for the US population (equivalent to a MOE of 345), 40% for all infants (equivalent to a MOE of 250) and 60% for children 1-2 yr (equivalent to a MOE of 167). Equivalent Tier 3 MOEs calculated by DPR for those subpopulations at the 99.9^{th} percentile were 164, 92 and 92 (NOEL = 1 mg/kg) and 41, 23 and 23 (LED_{10} = 0.25 mg/kg). DPR thus anticipates a higher level of risk from dietary exposure to carbaryl, regardless of the critical acute toxicity term used to calculate MOEs.

Chronic oral toxicity. USEPA did not estimate a chronic PoD for carbaryl, as it did not consider that carbaryl, with its rapid dissociation from the cholinesterase enzyme, posed a chronic risk (USEPA, 2007b). DPR's chronic oral LED_{10} of 0.5 mg/kg/day (ED_{10} = 1.7 mg/kg/day) was derived using benchmark dose methodology applied to cholinesterase inhibition data in the 1-year dog study of Hamada (1987). In any case, the lowest chronic MOE calculated by DPR, 763 for children 1-2 yr, was considered to represent a negligible risk due to chronic dietary exposure.

Oncogenicity. USEPA regards carbaryl as a "likely human carcinogen" (USEPA, 2007b). USEPA and DPR agreed that the formation of hemangiosarcomas in male mice, observed in the 2-year study of Hamada (1993b), was the most sensitive oncogenic endpoint (though DPR included hemangiomas). The 95% upper bound human equivalent potency slope values calculated by the two agencies were not similar, however, differing by a factor of 11.5-fold (USEPA: 8.75×10^{-4} mg/kg/day^{-1}; DPR: 1.01×10^{-2} mg/kg/day^{-1}). The major source of this discrepancy was almost certainly DPR's choice to eliminate the high dose in conducting the potency analysis (see discussion in sections IV.A.2 and V.A.2). USEPA's consequent risk value, 2.8×10^{-8} for the US population did not exceed the negligible risk standard of 10^{-6}. DPR's range of risk values, 2.54×10^{-6} - 3.83×10^{-6} for the adult populations analyzed, clearly did exceed that standard.

Reproductive and developmental toxicity. USEPA did not discuss either of the dog reproductive / developmental studies that evidenced toxicity (Smalley *et al.,* 1968; Immings *et al.,* 1969), nor was the dog metabolism study of Knaak *et al.* (1967) considered. A 1976 USEPA memo written by Dr. Neil Chernoff discounting the relevance of the dog data to human toxicology was assumed to constitute USEPA's current position on this issue and probably

underlaid their decision not to consider the dog studies in the RED (Chernoff [1976]). Dr. Chernoff's position is quoted below:

> "I feel that with the exception of the dog, in cases where severe maternal toxicity has not been observed there have been no consistent adverse reproductive or fetotoxic effects induced by carbaryl. The positive effects seen in the dog must be evaluated in light of its reported unusual metabolism. In the other species where positive effects have been shown, these effects must be considered in terms of maternal toxicity induced by the treatment, and the extremely high dose levels used. I feel that the use of such experiments which test for the maximum potential of a compound to induce effects is necessary to indicate types of effects to be looked for at lower dose levels (and such studies are regularly done in my laboratory). I do not feel that such studies should be afforded important consideration in the overall toxicological evaluation of safety for the continued use of carbaryl. I feel, therefore, that the evidence to date does not indicate that continued use of carbaryl would pose a reproductive or fetotoxic threat to man."

In contrast to USEPA, this assessment treats the dog studies at some length, as it did not consider the observed maternal, fetal and perinatal toxicity to be necessarily contravened by the available dog metabolism data.

In general, USEPA did not express a high level of concern about the potential for reproductive or developmental toxicity, as the NOELs in the contract studies that they examined were higher than the critical acute PoD of 1.1 mg/kg. Application of a Food Quality Protection Act safety factor of 1 reflected this view. DPR viewed reproductive toxicity mainly through the lens of epidemiologic studies, which indicated potential reproductive problems in males. DPR also considered the developmental toxicity evident in the beagle study discussed above to reflect a potential developmental risk.

Table V-1. Critical toxicity endpoints for carbaryl: USEPA *vs*. DPR

Study type	USEPA RED (USEPA, 2007a & 2007b)	DPR
Acute toxicity, regulator value #1	**Moser, 2007** **Acute toxicity - rat** LOEL value not determined (brain ChEI) LED_{10} = 1.1 mg/kg RfD & aPAD [a] = 0.01 mg/kg	**Robinson & Broxup, 1997** **Developmental ntx - rat** NOEL = 1 mg/kg RfD [b] = 0.01 mg/kg
Acute toxicity, regulator value #2	**Moser, 2007** **Acute toxicity - rat** LOEL value not determined (brain ChEI) LED_{10} = 1.1 mg/kg RfD & aPAD [a] = 0.01 mg/kg	**Robinson & Broxup, 1997** **Developmental ntx - rat** LOEL value not determined (increased slight hypotonic gait) LED_{10} = 0.25 mg/kg RfD [b] = 0.0025 mg/kg
Chronic toxicity	n/a (USEPA did not consider carbaryl to pose a chronic toxicity risk)	**Hamada, 1987** **1-year chronic - dog** LOEL = 3.1 mg/kg/day (brain / RBC / plasma ChEI) LED_{10} = 0.5 mg/kg/day RfD [1] = 0.005 mg/kg
Oncogenicity	**Hamada, 1993b** **2-year chronic / onco - mouse** Dose-dependent hemangiosarcomas 95% UB potency = 8.75×10^{-4}	**Hamada, 1993b** **2-year chronic / onco - mouse** Dose-dependent hemangiosarcomas / hemangiomas 95% UB potency = 1.01×10^{-2}

[a] *Abbreviations*: RfD, reference dose; aPAD, acute population adjusted dose. In the case of USEPA's carbaryl RED, the aPAD and RfD are equivalent (*i.e.*, both 1/100 of the acute LED_{10}).
[b] DPR reference doses are calculated in section VII.

VI. ISSUES RELATED TO THE FOOD QUALITY PROTECTION ACT

The Food Quality Protection Act of 1996 mandated the USEPA to "upgrade its risk assessment process as part of the tolerance setting procedures" (USEPA [1997a and b]). The improvements to risk assessment were based on recommendations made in the 1993 National Academy of Sciences report, "Pesticides in the Diets of Infants and Children" (NAS [1993]). The Act required an explicit finding that tolerances are safe for children. USEPA was required to use an extra 10-fold safety factor to take into account potential pre- and post-natal developmental toxicity and the completeness of the data, unless USEPA determined, based on reliable data, that a different margin would be safe. In addition, the USEPA must consider available information on: 1) aggregate exposure from all non-occupational sources; 2) effects of cumulative exposure to the pesticide and other substances with common mechanisms of toxicity; 3) the effects of *in utero* exposure; and 4) the potential for endocrine disrupting effects.

Aggregate exposure. Only a single route of exposure, oral, was considered for this document. Aggregate exposure (*i.e.*, exposure to a chemical by various routes) will be assessed at a later date when exposure estimates from other routes become available.

Cumulative exposure. USEPA is currently evaluating the potential for cumulative exposure to N-methyl carbamate pesticides. We will await the outcome of that evaluation before rendering a judgement on cumulative risk.

***In utero* effects.** Several lines of evidence suggest that carbaryl may have developmental effects: (1) epidemiologic studies in human populations have associated carbaryl exposure with sperm deficits or disorders (Savitz *et al.* [1997]; Wyrobek *et al.* [1981]; Xia *et al.* [2005]; Meeker *et al.* [2004a and 2004b]); (2) two older dog studies demonstrated developmental impacts when fetuses were exposed through the maternal diet (Smalley *et al.* [1968]; Immings *et al.* [1969]); (3) several animal studies evidenced direct carbaryl effects on sperm and/or spermatogenic tissue (Rybakova [1966]; Shtenberg and Rybakova [1968]; Pant *et al.* [1995, 1996]; Kitagawa *et al.* [1977]; (4) several *in vitro* genotoxicity studies showed positive effects of carbaryl; and (5) a guideline rat reproductive study showed increased pup mortality, reduced body weights and delayed developmental indices at 1500 ppm (92-136 mg/kg/day) and increased F_2 mortality, pnd 0-4, at 300 (5-6 mg/kg/day) and 1500 ppm (Tyl *et al.* [2001]). However, the relevance of the dog system to humans has been questioned in regard to the fetal and developmental effects (see discussions in sections III.G.2.c., IV.A.1.a, V.A.1.a, V.A.1.c., V.A.1.d. and V.D.). Neither of the guideline developmental toxicity studies (Tyl *et al.* [1999], in rabbits; Repetto-Larsay [1998], in rats), showed developmental effects of this nature in rats or rabbits, though the issue of spermatogenic defects was not specifically addressed. Finally, no direct evidence of birth defects has been reported in human populations, despite many years of carbaryl usage. In view of these competing considerations, this assessment does not make a recommendation regarding reproductive or developmental toxicity. At the very least, this will await the submission of more contemporary studies in the dog system.

Endocrine effects. The mechanisms by which carbaryl disrupts canine pregnancies or induces testicular toxicity are unknown, though it remains possible that endocrine pathways are involved. Nonetheless, the extent of endocrine involvement, if any, in such effects should be approached with specifically designed studies.

VII. REFERENCE DOSES (RfDs)

Oral doses of carbaryl below a calculated reference dose (RfD) were considered unlikely to pose a risk to human health. RfDs were calculated for acute and chronic dietary exposure scenarios by dividing the critical oral NOELs by an uncertainty factor of 100. All of the uncertainties that accompanied selection of this endpoint were applicable to this calculation (see section V.A.). Potential exposures sustained under occupational scenarios, either by the dermal or inhalation routes, were outside the scope of this analysis. The RfDs calculated below were most relevant to the general population exposed through the diet. Two such values were calculated for acute oral exposure, reflecting the critical acute NOEL of 1 mg/kg and the critical acute LED_{10} of 0.25 mg/kg.

1. Critical acute NOEL = 1 mg/kg

RfD = critical acute NOEL ÷ 100
critical acute oral NOEL = 1 mg/kg
RfD_{acute} = 1 mg/kg ÷ 100 = **0.01 mg/kg**

2. Critical acute LED_{10} = 0.25 mg/kg

RfD = critical acute LED_{10} ÷ 100
critical acute oral LED_{10} = 0.25 mg/kg
RfD_{acute} = 0.25 mg/kg ÷ 100 = **0.0025 mg/kg**

3. Critical chronic oral NOEL = 0.5 mg/kg/day

RfD = critical chronic oral NOEL ÷ 100
critical chronic oral NOEL = 0.5 mg/kg/day
$RfD_{chronic}$ = 0.5 mg/kg/day ÷ 100 = **0.005 mg/kg/day**

In Table VII-1, the calculated oral RfDs for carbaryl are compared with the anticipated dietary exposure ranges for the various subpopulations. If the RfD_{acute} is established using the critical acute NOEL of 1 mg/kg (RfD_{acute} = 0.01 mg/kg), acute dietary exposures calculated using the point estimate approach at the 95th percentile exceeded the RfD_{acute} for all infants, nursing infants <1 yr, non-nursing infants <1 yr, children 1-2 yr and children 3-5 yr. Using the Monte Carlo approach at the 99.9th percentile, the RfD_{acute} was exceeded for all infants, non-nursing infants <1 yr and children 1-2 yr. If, on the other hand, the RfD_{acute} is established using the critical acute LED_{10} of 0.25 mg/kg (RfD_{acute} = 0.0025 mg/kg), acute dietary exposures calculated using both the point estimate approach at the 95th percentile and the Monte Carlo approach at the 99.9th percentile exceeded the RfD_{acute} for all of the subpopulations yielding sufficient user day data (see footnote to Table VII-7). Thus the exposure data combined with RfD_{acute} value suggest that exposure mitigation for acute effects should be considered.

By contrast, the highest chronic dietary exposure estimate, which was recorded for non-nursing infants <yr, was only 12% of the $RFD_{chronic.}$ By this measure, chronic exposure to carbaryl does not pose a non-oncogenic risk to any sub-population and does not currently warrant mitigation.

Table VII-1. Oral reference doses (RfDs) and anticipated dietary exposures to carbaryl

Exposure time and species	Endpoint	LOEL and NOEL (or LED)	RfD	Anticipated exposures
Acute NOEL rat gavage dvpmt. ntx. study, gd 6 - ppd 10 (Robinson & Broxup, 1997)	cholinergic signs and body weight gain decrements	**LOEL** 10 mg/kg **NOEL** 1 mg/kg	0.01 mg/kg	**dietary, 95th percentile, Tier 2 (PE)** [b] 0.004087 - 0.014953 mg/kg/day **dietary, 99.9th percentile, Tier 3(MC)** [b] 0.003564 - 0.012796 mg/kg/day
Acute LED_{10} rat gavage dvpmt. ntx. study, gd 6 - ppd 10 (Robinson & Broxup, 1997)	slight hypotonic gait	**LOEL** not determined [a] **LED_{10}** 0.25 mg/kg	0.0025 mg/kg	**dietary, 95th percentile, Tier 2 (PE)** [b] 0.004087 - 0.014953 mg/kg/day **dietary, 99.9th percentile, Tier 3(MC)** [b] 0.003564 - 0.012796 mg/kg/day
Chronic NOEL dog 1-yr dietary study (Hamada, 1987)	brain ChEI	**LOEL** 3.4 mg/kg/day **LED_{10}** 0.5 mg/kg/day	0.005 mg/kg/day	**dietary (chronic)** [b] 0.000225 - 0.000655 mg/kg/day

[a] An appearance of slight hypotonic gait at 10 mg/kg was certain, though less certain at 1 mg/kg. As a consequence, these data were subjected to benchmark dose analysis.
[b] PE, point estimate; MC, Monte Carlo estimate. The dietary subpopulations examined are those covered in the DEEM-FCID® dietary analysis. The dietary exposure values were taken from Tables IV-3 and IV-4. Two subpopulations - females 13+ pregnant / not nursing and females 13+ nursing - were excluded from this comparison due to an insufficient number of available user days for analysis.

VIII. TOLERANCE ASSESSMENT

A tolerance is the legal maximum residue concentration of a pesticide, which may be present in or on a raw agricultural commodity or processed food. USEPA is reponsible under the Federal Food, Drug and Cosmetic Act (FFDCA) for setting tolerances for pesticide residues (Section 408). The tolerances are established at levels necessary for the maximum application rate and frequency, and are not expected to produce deleterious health effects in humans from chronic dietary exposure (USEPA, 1991).

The data requirements for the registration of pesticides and for establishment of tolerances include: (1) residue chemistry (including measured residue levels from field studies), (2) environmental fate, (3) toxicology, (4) product performance (*i.e.*, efficacy), and (5) product chemistry. The field studies must reflect the proposed uses with respect to the rate and mode of application, the number and timing of applications and the proposed formulations (USEPA, 1982).

In 1996, the Food Quality Protection Act (FQPA) amended the overall regulation of pesticide residues under FIFRA and FFDCA (USEPA, 1997a). Significantly, the Delaney Clause, which prohibited residues of cancer-causing pesticides in processed foods, was removed. However, FQPA requires scientific evidence showing that tolerances are safe for infants and children. USEPA must consider applying an additional safety factor of up to 10 to take into account potential pre- and post-natal developmental toxicity and completeness of the database.

FQPA also requires USEPA to reassess existing tolerances and tolerance exemptions for active and inert ingredients by 2006 (USEPA, 1997b). Tolerance reassessments had previously been executed as part of USEPA's re-registration and Special Review processes. All label-use commodities are evaluated using a tiered approach similar to that used for the general dietary assessments.

In California, Assembly Bill 2161 (The Food Safety Act) requires DPR to "conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides" (Bronzan and Jones [1989]). In situations whereby "any pesticide represents a dietary risk that is deleterious to health of humans, the DPR shall prohibit or take action to modify that use or modify the tolerance".

A. ACUTE EXPOSURE

An acute tolerance assessment was conducted for each "high-contributor" commodity, defined for this assessment as those commodities providing greater than 5% of the total carbaryl consumption as indicated by the Critical Exposure Contribution analysis for infants and children 1-2 yr using the DEEM-FCID® Tier 2 / point estimate approach. These commodities included blueberries, peaches, asparagus, apricots, blackberries and apples. The consumption database came from the USDA Continuing Survey of Food Intakes of Individuals 1994-1998 (CSFII). The acute tolerance assessment did not address simultaneous consumption of multiple commodities at tolerance levels. The probability of consuming multiple commodities at such levels significantly decreases as the number of commodities included in the assessment increases. Consumption of even two commodities at tolerance was considered unlikely (and in any case, the MOEs were below 100 for single commodities; see below).

The exposure levels and MOE values at the 95th exposure percentile for each analyzed commodity is shown in Table VIII-1. It should be noted that the consumption patterns for a given subpopulation is better represented when the survey sample size is sufficiently large (*i.e.*, >100 user days). Data from subgroup-commodity pairs with less than this amount were considered to be unreliable. Despite this caveat, MOEs of less than 100 were indicated for every commodity examined when there were sufficient user days available for analysis, regardless of whether 1 mg/kg or 0.25 mg/kg was used as the toxicology term. Thus carbaryl tolerances should be reevaluated, as residues at the current tolerance levels result in inadequate MOEs. The data are summarized in Table VIII-1.

Table VIII-1. Acute dietary margins of exposure for high contributing commodities (>5%) at the carbaryl tolerance level; 95th percentile user days

Population subgroup	Commodity and tolerance [a]					
	Blueberry 10 ppm	**Peach 10 ppm**	**Asparagus 15 ppm**	**Apricot 10 ppm**	**Blackberry 10 ppm**	**Apple 12 ppm**
1. US population						
MOE (95th percentile)	304 / 76	40 / 10	12 / 3	116 / 29	484 / 121	4 / 1
exposure (mg/kg)	.003286	.023238	.063893	.008479	.002049	.148859
unweighted user days	10472	11921	239	4723	3944	17358
2. Western region						
MOE (95th percentile)	336 / 84	36 / 9	20 / 5	84 / 21	420 / 105	4 / 1
exposure (mg/kg)	.002943	.025162	.047470	.011597	.002368	.150441
unweighted user days	2410	2854	65	1274	1055	4064
3. Hispanics						
MOE (95th percentile)	490 / 124	32 / 8	20 / 5	140 / 35	664 / 166	4 / 1
exposure (mg/kg)	.002013	.030979	.047618	.006945	.001503	.187283
unweighted user days	1051	1282	9	520	438	2066
4. Non-hispanic whites						
MOE (95th percentile)	268 / 67	44 / 11	12 / 3	92 / 23	416 / 104	4 / 1
exposure (mg/kg)	.003727	.022555	.064136	.010575	.002389	.141253
unweighted user days	7766	8687	214	3232	2630	12124
5. Non-hispanic blacks						
MOE (95th percentile)	492 / 123	44 / 11	4 / 1	688 / 172	1464 /366	4 / 1
exposure (mg/kg)	.002022	.021727	.209938	.001453	.000683	.156632
unweighted user days	1243	1458	7	772	713	2350
6. Non-hispanic / non- white / non-black						
MOE (95th percentile)	272 / 68	48 / 12	32 / 8	88 / 22	832 / 208	4 / 1
exposure (mg/kg)	.003640	.019714	.029474	.011033	.001198	.172082
unweighted user days	412	494	9	199	163	818
7. All infants						
MOE (95th percentile)	28 / 7	4 / 1	4 / 1	8 / 2	36 / 9	2 / 0
exposure (mg/kg)	.035386	.155827	.148701	.086223	.026414	.429168
unweighted user days	202	439	3	197	46	1149
8. Nursing infants <1 yr						
MOE	20 / 5	4 / 1	no	12 / 3	48 / 12	0
exposure (mg/kg)	.046887	.155543	exposure	.068533	.020003	.379791
unweighted user days	44	88		38	13	213
9. Non-nursing infants <1 yr						
MOE (95th percentile)	28 / 7	4 / 1	4 / 1	8 / 2	36 / 9	2 / 0
exposure (mg/kg)	.033858	.155261	.148701	.092135	.026402	.430391
unweighted user days	158	351	3	156	33	936

10. Females 13+ (preg./not lact.) MOE (95th percentile) exposure (mg/kg) unweighted user days	448 / 112 .002224 33	32 / 8 .030187 35	no exposure	48 / 12 .019625 10	256 / 64 .003873 8	4 / 1 .130641 48
11. Females 13+ (lactating) MOE (95th percentile) exposure (mg/kg) unweighted user days	696 / 174 .001429 22	56 / 14 .017311 22	no exposure	56 / 14 .017002 8	972 / 243 .001025 6	12 / 3 .064994 36
12. Children 1-2 yr MOE (95th percentile) exposure (mg/kg) unweighted user days	160 / 40 .006247 1241	8 / 2 .086612 1420	4 / 1 .187562 19	24 / 6 .036493 552	324 / 81 .003085 445	2 / 0 .436698 2655
13. Children 3-5 yr MOE (95th percentile) exposure (mg/kg) unweighted user days	180 / 45 .005462 3103	20 / 5 .048039 3311	4 / 1 .148065 23	128 / 32 .007735 1529	404 / 101 .002472 1391	3 / 0 .294408 5347
14. Children 6-12 yr MOE (95th percentile) exposure (mg/kg) unweighted user days	280 / 70 .003559 1470	36 / 9 .025099 1541	4 / 1 .209485 13	216 / 54 .004557 716	516 / 129 .001931 671	4 / 1 .133644 2112
15. Youth 13-19 yr MOE (95th percentile) exposure (mg/kg) unweighted user days	404 / 101 .002453 584	56 / 14 .017622 593	20 / 5 .043737 4	332 / 83 .002987 281	596 / 149 .001671 256	8 / 2 .085884 731
16. Adults 20-49 yr MOE (95th percentile) exposure (mg/kg) unweighted user days	344 / 86 .002875 1841	52 / 13 .018096 1920	12 / 3 .068874 59	144 / 36 .006894 621	564 / 141 .001771 497	16 / 4 .054440 2372
17. Adults 50+ yr MOE (95th percentile) exposure (mg/kg) unweighted user days	384 / 96 .002589 2031	52 / 13 .018106 2697	16 / 4 .053899 118	88 / 22 .010982 830	552 / 138 .001809 638	20 / 5 .045579 2992
18. Females 13-49 yr MOE (95th percentile) exposure (mg/kg) unweighted user days	348 / 87 .002865 1245	48 / 12 .020126 1340	12 / 3 .068682 29	200 / 50 .004984 501	648 / 162 .001536 409	16 / 4 .062256 1634
19. Males/Females 16-70 yr MOE (95th percentile) exposure (mg/kg) unweighted user days	344 / 86 .002883 3588	56 / 14 .017360 4064	16 / 4 .058082 137	160 / 40 .006231 1307	504 / 126 .001982 1058	16 / 4 .052875 4810

Note: An acute tolerance assessment was conducted for carbaryl residues on each commodity which registered higher than 5% dietary contribution in the Tier II DEEM-FCID® point estimate for infants and for children 1-2 yr. The carbaryl residue level for each commodity was set at the tolerance. The DEEM-FCID® program was used with the following input parameters: (1) USDA CSFII 1994-1998, and (2) an acute

NOEL of 1 mg/kg or an acute LED_{10} 0.25 mg/kg. The first value in the top line of each box represents the MOE calculated with the 1 mg/kg NOEL, the second with the 0.25 mg/kg LED_{10}. The precise MOE may differ slightly from the quotient of the NOEL (or LED) ÷ exposure dose due to rounding. This may be accented with the 1 mg/kg MOE because it was calculated by multiplying the DEEM-derived 0.25 mg/kg MOE by 4. The acute dietary exposure was calculated at the 95th percentile of user days for all of the population subgroups examined in the dietary assessment.

[a] Tolerances were listed in the Federal Register, Vol. 73, No. 176, Sept. 10, 2008.

B. CHRONIC EXPOSURE

A chronic exposure assessment using residue levels set to the established carbaryl tolerances was not attempted. It was highly improbable that single or multiple commodities containing pesticide levels at tolerance would be consumed habitually. This conclusion was supported by data from both the federal and DPR pesticide monitoring programs which indicated that only a minute fraction of all sampled commodities contained residue levels at or above the established tolerance.

IX. CONCLUSIONS

A dietary risk characterization was carried out for the insecticide carbaryl. It includes a complete toxicity profile and summaries of carbaryl's environmental fate, current uses and illness / injury reports for the years 1992-2007, in addition to a dietary exposure assessment and risk characterization for 19 different human subpopulations. Laboratory investigations indicated a potential for toxicity under various exposure routes and times of exposure, including some that indicate that carbaryl is oncogenic. The document also reviews several laboratory and epidemiologic studies that suggest that carbaryl may have adverse impacts on reproduction and/or development. Acute and chronic dietary exposure scenarios were considered for the risk characterization aspect of this document. Because the critical LED_{10}s were based on laboratory animal studies, a margin of exposure (MOE) of 100 was considered sufficiently protective of human health.

Critical NOELs, LEDs and cancer potency values. The following values, based on laboratory animal studies, were established for carbaryl (including two acute oral values from the same study representing endpoints of stronger or weaker experimental support):

♦ Acute oral NOEL: **1 mg/kg** - based on the appearance of cholinergic signs detected in FOB testing (slight tremors, slight hypotonic gait, slight ataxic gait and pinpoint pupils) and body weight gain decrements at 10 mg/kg.

♦ Acute oral LED_{10}: **0.25 mg/kg** - based on induction of slight hypotonic gait in a rat gavage study over the dose range of 0.1 - 10 mg/kg.

♦ Chronic oral LED_{10}: **0.5 mg/kg/day** - based on inhibition of brain cholinesterase activity in a 1-yr dog dietary study.

♦ Human equivalent cancer potency value: 1.01×10^{-2} mg/kg/day^{-1} (the 95% upper bound estimate) and 4.29×10^{-4} mg/kg/day^{-1} (maximum likelihood estimate) - based on hemangiosarcomas and hemangiomas in both sexes in a mouse two-year feeding study.

♦ Reproductive and developmental toxicity: Evidence originating in non-guideline studies, both of an epidemiologic and laboratory animal nature, suggested the possibility that carbaryl could have reproductive and/or developmental impacts. However, based on insufficient data, this assessment does not present a risk evaluation regarding reproductive or developmental toxicity.

Dietary risk characterization. A dietary exposure and risk evaluation was conducted for 19 subpopulations, and included all commodities for which carbaryl tolerances exist, in addition to drinking water.

♦ Acute toxicity (NOEL = 1 mg/kg): Margins of exposure (MOEs) based on the highest residue determination for each commodity (Tier 2) were below the health protective standard of 100 for several subpopulations at the 95th user day percentile. At the 99th user day percentile, most populations showed MOEs less than 100, reaching as low as 24 for all infants and non-nursing infants <1 yr. A refined Tier 3 / Monte Carlo analysis was triggered because of these low values.

There were no subpopulations exhibiting MOEs below 100 at the 95 or 99th user day percentiles using Monte Carlo analysis (Tier 3). However, at the 99.9th percentile, three subpopulations exhibited MOEs below 100: all infants (MOE=91), non-nursing infants <1 yr (MOE=76) and children 1-2 (MOE=92).

♦ Acute toxicity (LED_{10} = 0.25 mg/kg): Margins of exposure (MOEs) based on the highest residue determination for each commodity (Tier 2) were below the health protective standard of 100 for all subpopulations at the 95th and 99th user day percentiles. The lowest MOEs at any percentile were found among infants, reaching 6 at the 99th percentile and 16 at the 95th percentile for non-nursing infants <1 yr. For all infants these values were 6 and 19, respectively, while for nursing infants <1 yr they were 9 and 17. Because these values were so low, Tier 3 / Monte Carlo analysis was triggered.

Monte Carlo-derived MOEs at the 99.9th percentile fell below the health-protective standard of 100 for almost all subpopulations. As with the point estimate data, the lowest MOEs occurred among infants and young children 1-2 years, reaching 19 for non-nursing infants <1 yr at the 99.9th percentile. Even at the 99th percentile there were many subpopulations registering MOEs below 100, including one (non-hispanic non-white non-black) that included adults. All subpopulations showed MOEs greater than 100 at the 95th percentile in the Monte Carlo analysis.

♦ Chronic toxicity: As with acute exposure, the highest predicted chronic exposure was to children 1-2 yr, generating a MOE of 763. A chronic dietary health concern was, consequently, not indicated for carbaryl.

♦ Oncogenic risk: Oncogenic risk ranged between 2.54×10^{-6} and 3.83×10^{-6} for the adult populations analyzed. As this range was greater than the health-protective target value of 10^{-6}, dietary consumption of carbaryl was considered to constitute an oncogenic risk.

Reference doses (RfDs). Oral doses of carbaryl below a calculated RfD were considered unlikely to pose a risk to human health. RfDs were calculated for acute and chronic dietary exposure scenarios by dividing the critical oral NOEL or LED_{10} values by an uncertainty factor of 100 to account for variations in sensitivity in animal and human populations.

♦ RfD_{acute} = 0.01 mg/kg if the acute NOEL of 1 mg/kg is used in the RfD calculation. Acute dietary exposures calculated using the point estimate approach at the 95th percentile exceeded the RfD_{acute} for all infants, nursing infants <1 yr, non-nursing infants <1 yr, children 1-2 yr and children 3-5 yr. Using the Monte Carlo approach at the 99.9th percentile, the RfD_{acute} was exceeded for all infants, non-nursing infants <1 yr and children 1-2 yr. These data suggest that mitigation of carbaryl in food sources may be warranted.

♦ RfD_{acute} = 0.0025 mg/kg if the acute LED_{10} of 0.25 mg/kg is used in the RfD calculation. Acute dietary exposures calculated using both the point estimate approach at the 95th percentile and the Monte Carlo approach at the 99.9th percentile exceeded the RfD_{acute} for all of the subpopulations that yielded sufficient user day data. These data also suggest that mitigation of carbaryl in food sources may be warranted.

♦ $RfD_{chronic}$ = 0.005 mg/kg/day. This value exceeded all chronic dietary exposure estimates.

Tolerance assessment. A separate acute tolerance assessment was conducted for each "high-contributor" commodity, defined for the tolerance assessment as those commodities providing greater than 5% of the total carbaryl consumption in the Tier 2 point estimate calculations at the 95th percentile as indicated by the Critical Exposure Contribution analysis for infants and children 1-2 yr. These commodities included blueberries, peaches, asparagus, apricots, blackberries and apples. MOEs of less than 100 were indicated for every commodity examined where there were sufficient user days available, regardless of which critical acute toxicity value was used in the DEEM calculation. Consequently, many tolerance values may warrant reevaluation.

A chronic exposure assessment using residue levels set to the established carbaryl tolerances was not attempted, as it was highly improbable that single or multiple commodities containing pesticide levels at tolerance would be consumed habitually.

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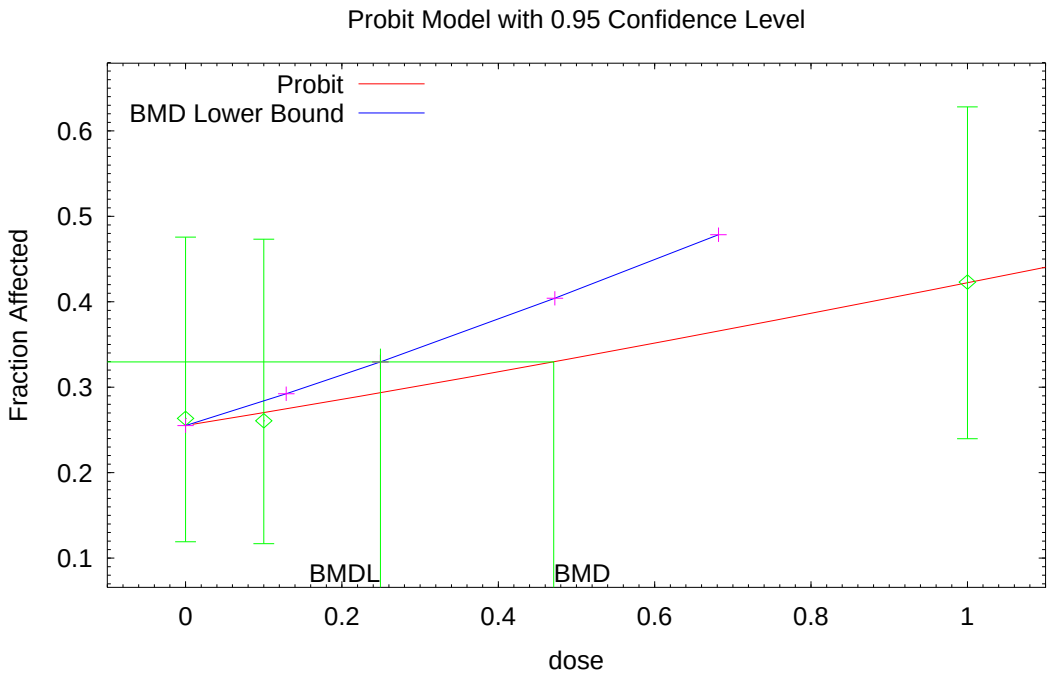
Appendix I. Benchmark dose extrapolation for induction of slight hypotonic gait in pregnant Sprague-Dawley rats (Robinson and Broxup, 1997)



Robinson and Broxup (1997) rat acute neurotoxicity study with carbaryl
Slight hypotonic gait data in males (top dose deleted)
Probit model; slope parameter not restricted
Risk type: "Extra risk"

10% benchmark response:

==========

Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
Input Data File:
C:\BMDS\DATA\CARBARYL_HYPOTONIC_GAIT_NORMALIZED_MEAN_GD_6_TO_20.(d)
Gnuplot Plotting File:
C:\BMDS\DATA\CARBARYL_HYPOTONIC_GAIT_NORMALIZED_MEAN_GD_6_TO_20.plt
Wed May 06 09:39:08 2009

==========

BMDS MODEL RUN

~~~~~~~~~~

The form of the probability function is:

P[response] = CumNorm(Intercept+Slope*Dose),
~~~~~~~~~~

where CumNorm(.) is the cumulative normal distribution function

Dependent variable = COLUMN3
Independent variable = COLUMN1
Slope parameter is not restricted

Total number of observations = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
background = 0 Specified
intercept = -0.648276
slope = 0.454457

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.65
slope	-0.65	1

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	-0.658436	0.198162
slope	0.461902	0.326088

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-47.3395			
Fitted model	-47.3503	0.0215168	1	0.8834

Reduced model -48.3536 2.02813 2 0.3627

AIC: 98.7005

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.2551	6.582	7	26	0.0983
0.1000	0.2702	6.944	7	26	-0.1083
1.0000	0.4221	10.974	11	26	0.01013

Chi-square = 0.02 DF = 1 P-value = 0.8834

Benchmark Dose Computation

Specified effect = 0.1

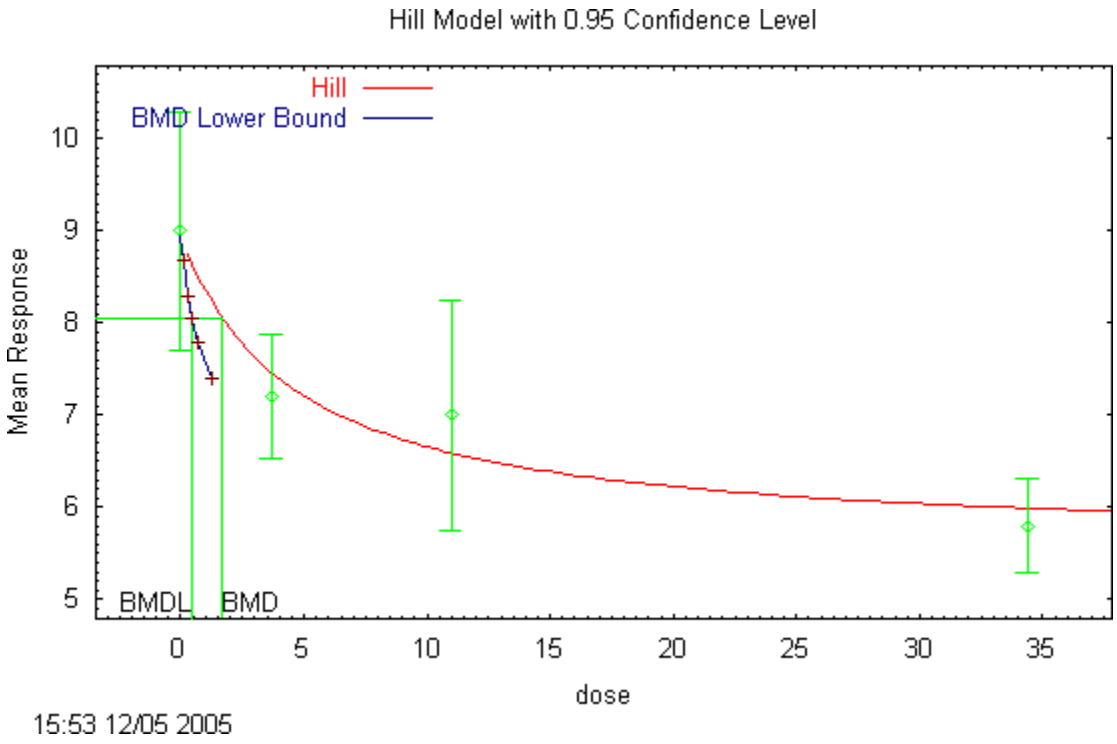
Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.470798

BMDL = 0.249141

Appendix II. Benchmark dose extrapolation for brain cholinesterase inhibition in female dogs after 52 weeks of exposure to dietary carbaryl (Hamada, 1987)



Hamada (1987) dog 1-yr dietary study with carbaryl
Brain cholinesterase data in females
Hill model, n>1
Risk type: "Relative risk"

10% benchmark response:

==========

Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
Input Data File: D:\BMDS\UNSAVED1.(d)
Gnuplot Plotting File: D:\BMDS\UNSAVED1.plt
Mon Dec 05 15:53:22 2005

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BMDS MODEL RUN

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The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
~~~~~~~~~~

Independent variable = dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	0.670558
rho =	0 Specified
intercept =	9
v =	-3.2
n =	1.2784
k =	3.28889

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	rho	intercept	v	k
alpha	1	0	0	0	0
rho	0	1	0	0	0
intercept	0	0	1	0	0
v	0	0	0	l	0
k	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.812608	1
rho	0	1
intercept	8.95158	1

v	-3.35799	1
n	1	NA
k	4.65005	1

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	6	9	1.23	8.95	0.901	0.0537
3.7	6	7.2	0.64	7.46	0.901	-0.292
11	6	7	1.19	6.59	0.901	0.453
34.4	6	5.8	0.48	5.99	0.901	-0.215

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-8.444034	5	26.888069
A2	-5.016409	8	26.032817
fitted	-9.509932	4	27.019865
R	-21.130889	2	46.261778

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)

Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	32.229	6	<.0001
Test 2	6.85525	3	0.07666
Test 3	2.1318	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 1.69014

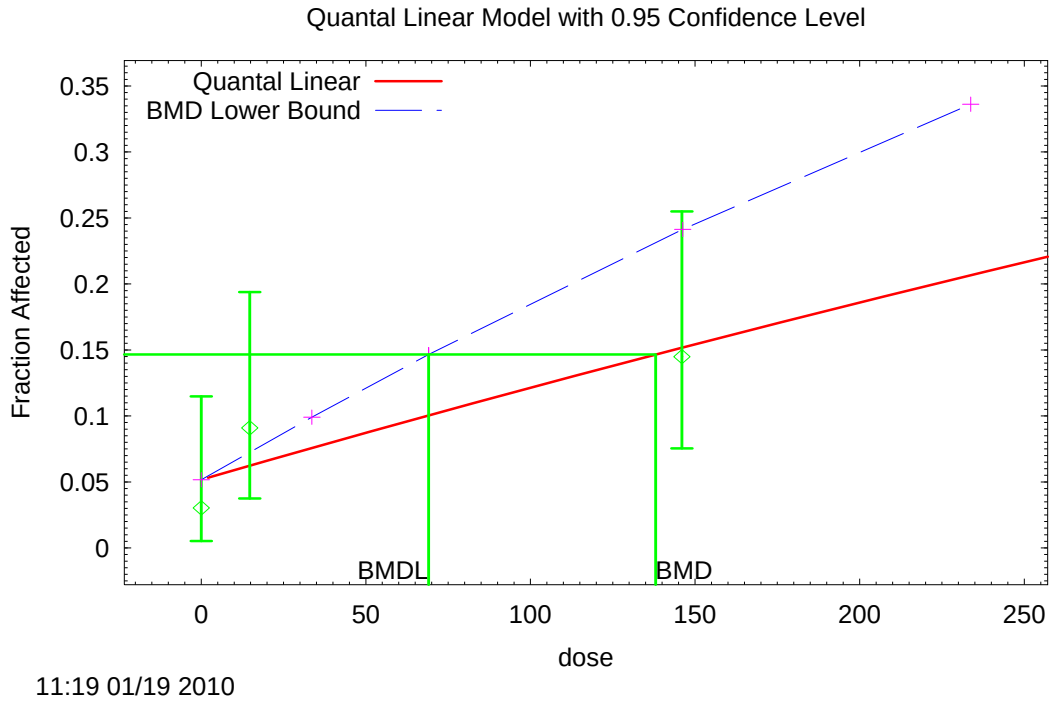
BMDL = 0.46739

Appendix III. Benchmark dose extrapolation for hemangiosarcoma / hemangioma data in male mice (Hamada, 1993b)

Quantal linear model; top dose deleted (10% benchmark dose lines included in graph)

To calculate the oncogenic potency at a risk of 10^{-6}:

risk ÷ dose (*i.e.*, LED_{10} x 10^{-5}) = potency
10^{-6} ÷ (69.045 x 10^{-5}) = 1.45 x 10^{-3} $(mg/kg/day)^{-1}$



Hamada (1993b) mouse 2-yr dietary oncogenicity study with carbaryl
Hemangiosarcoma / hemangioma data in males
Quantal linear model
Risk type: "Extra risk"
==========

Quantal Linear Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
Input Data File: C:\BMDS\DATA\CARBARYL_MOUSE_ONCO_HAMADA_1993.(d)
Gnuplot Plotting File: C:\BMDS\DATA\CARBARYL_MOUSE_ONCO_HAMADA_1993.plt
Tue Jan 19 11:19:27 2010

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BMDS MODEL RUN
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The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose)]

Dependent variable = COLUMN3
Independent variable = COLUMN1

Total number of observations = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.0373134
Slope = 0.00085274
Power = 1 Specified

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.52
Slope	-0.52	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.0516686	0.0221186
Slope	0.000763212	0.000410313

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value

Full model	-57.6212			
Fitted model	-58.4014	1.5605	1	0.2116
Reduced model	-60.6016	5.96091	2	0.05077

AIC: 120.803

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0517	3.410	2	66	-0.7841
14.7300	0.0623	4.110	6	66	0.9628
145.9900	0.1517	10.464	10	69	-0.1559

Chi-square = 1.57 DF = 1 P-value = 0.2108

Benchmark Dose Computation

Specified effect = 0.1

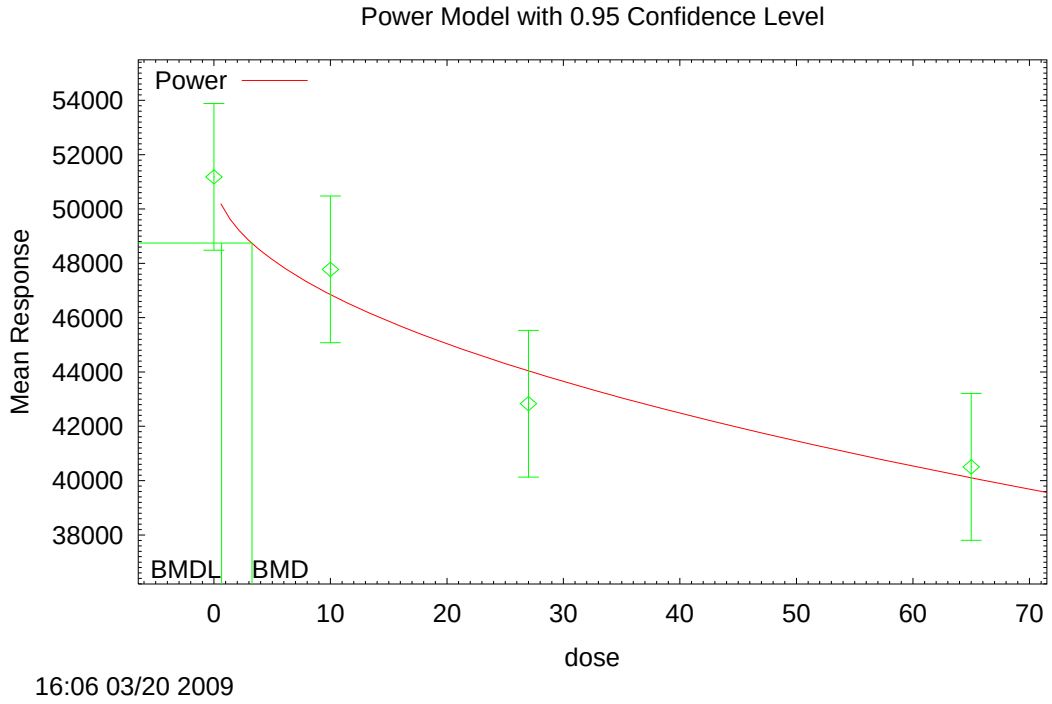
Risk Type = Extra risk

Confidence level = 0.95

BMD = 138.049

BMDL = 69.045

Appendix IV. Benchmark dose extrapolation for brain cholinesterase inhibition data in female rats following acute inhalation exposure (Weinberg, 2008)



Weinberg (2008) rat acute inhalation toxicity study with carbaryl
Brain cholinesterase inhibition data in males
Power model
Risk type: "Relative risk"

```
================================================================================
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                  Fri Mar 20 16:06:46 2009
================================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = COLUMN1
```

rho is set to 0
The power is not restricted
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 4.73226e+006
rho = 0 Specified
control = 51181
slope = -813.168
power = 0.616791

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	-0.096	0.15	0.14
rho	-1	1	0.096	-0.15	-0.14
control	-0.096	0.096	1	-0.63	-0.49
slope	0.15	-0.15	-0.63	1	0.97
power	0.14	-0.14	-0.49	0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	4.40772e+006	2.01428e+008
rho	0	4.25088
control	51313.4	925.597
slope	-1432.98	732.141
power	0.493027	0.11873

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	5.12e+004	2.41e+003	5.13e+004	2.1e+003	-0.0631
10	5	4.78e+004	2.97e+003	4.69e+004	2.1e+003	0.439
27	5	4.28e+004	1.4e+003	4.4e+004	2.1e+003	-0.573
65	5	4.05e+004	1.53e+003	4.01e+004	2.1e+003	0.197

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-161.467702	5	332.935403
A2	-159.577513	8	335.155026
fitted	-162.988677	4	333.977355
R	-179.187770	2	362.375539

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	39.2205	6	<.00001
Test 2	3.78038	3	0.2862
Test 3	3.04195	1	0.08114

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 13.294

BMDL = 5.51202

Appendix IV (continued). Summary of benchmark dose analysis for brain cholinesterase inhibition following acute inhalation exposure (2nd cohort) (Weinberg, 2008)

Relative risk, values expressed as mg/m^3

	ED05	LED05	ED10	LED10
Male brain				
Linear	n/a	n/a	n/a	n/a
Polynomial	n/a	n/a	n/a	n/a
Power (power >1)	12.95	10.82	15.90	21.65
Power (unrestricted power)	8.31	3.04	20.40	11.56
Hill (n>1)	14.51	10.46	19.47	12.91
Hill (n unrestricted)	14.51	10.46	19.47	12.91
Female brain				
Linear	n/a	n/a	n/a	n/a
Polynomial	n/a	n/a	n/a	n/a
Power (power >1)	15.93	12.00	31.87	26.00
Power (unrestricted power)	3.26	0.65	13.29	5.51
Hill (n>1)	8.13	3.72	14.15	9.29
Hill (n unrestricted)	8.13	3.15	14.15	9.45

Appendix V.

DEEM-FCID® Acute Dietary Exposure Assessment and Risk Calculations - Point Estimate (Tier 2) and Monte Carlo (Tier 3)

Appendix is available by request at <publicrecords@cdpr.ca.gov>

Appendix VI.

Residue Data Files (RDFs) for the Acute Monte Carlo Analysis

Appendix is available by request at <publicrecords@cdpr.ca.gov>

Appendix VII.

DEEM-FCID® Chronic and Oncogenic Dietary Exposure Assessment and Risk Calculations

Appendix is available by request at <publicrecords@cdpr.ca.gov>

Appendix VIII. The Environmental Fate of Carbaryl

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I. Introduction

Carbaryl (1-naphthyl-*N*-methyl carbamate; Fig. 1) is a carbamate insecticide introduced in 1956 by Union Carbide Corporation. The insecticide is used worldwide and is a substitute for some organochlorine pesticides (Ribera et al., 2001). Carbaryl controls a broad spectrum of insects on more than 120 different crops (Ware, 2000). It has also been used to prevent bark beetle attacks in pine trees (Hastings et al., 2001) and as a general garden insecticide (Ware, 2000). In 2004, approximately 110,000 kg of the insecticide was applied in California alone (CDPR, 2004). Annual use in the U.S. is reported to be 4.5-6.8 million kg (Cox, 1993). Several trade names are associated with carbaryl; the most common being Sevin®. Active ingredient (a.i.) use rates for carbaryl range from 0.57-4.5 kg/ha (Rajagopal et al., 1984). It is available in the form of a wettable powder, pellets, granules, suspensions, and solutions. The insecticide is the second most widely detected insecticide in surface waters in the U.S. (Martin et al., 2003).

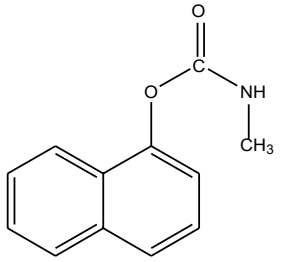

Fig. 1. Chemical structure of carbaryl.

II. Chemistry

Carbaryl, like most carbamates, inhibits the enzyme that degrades acetylcholine - acetylcholinesterase. Inhibition of this enzyme promotes the buildup of acetylcholine at synapses resulting in uncontrolled movement, paralysis, convulsions, and possible death (Tomlin, 2000).

The physical chemical properties of carbaryl are listed in Table 1. Carbaryl is a low molecular weight compound that is moderately soluble in water and does not readily volatilize. The compound is not compatible with alkaline materials such as lime (Tomlin, 2000).

Table 1. Physical-chemical properties of carbaryl.

Pure physical state [a]		Colorless or tan crystal
Chemistry Abstracts Service registry number (CAS #) [b]		63-25-2
Molecular weight (g/mol) [a]		201.2
Molecular formula [a]		$C_{12}H_{11}NO_2$
Melting point (°C) [a]		142
Vapor pressure (mPa at 23.5°C) [a]		0.041
Octanol-water partition coefficient (log K_{ow}) [a]		2.36
Density (20°C) [a]		1.23
Henry's law constant (atm m^3 g/mol at 25°C) [a]		$2.74x10^{-9}$
Organic-carbon normalized partition coefficient (K_{oc}) [b]		290
λ_{max} (nm) [c]		280
Water solubility (mg/L)	20°C [a]	120
	25°C [d]	104
	40°C [e]	40

[a] Tomlin, 2003; [b] Phillips and Bode, 2004 [c] Sheng et al., 2001; [d] Arroyo et al., 2004; [e] Meister, 2001.

III. Chemodynamics

A. Air

The low vapor pressure measured for carbaryl makes the possibility of volatilization unlikely (Table 1). Additionally, its low Henry's law constant suggests that it will not volatilize from aqueous solutions (Table 1). However, carbaryl could become airborne from binding to particulates or as a spray drift immediately following application. Drift monitoring from aerial spraying of carbaryl at a rate of 2250 g a.i./ha on a Vermont apple orchard revealed concentrations of 0.70-7.20 μg/plate (1 mm thick Teflon sheet covered the 15 cm diameter petri plate), which corresponds to 0.4-4.1 g a.i./ha, as far out as 305 meters with 8-12 km/h winds (Currier et al. ,1982). Higher concentrations (481 μg/plate) were observed at 76 meters

downwind and 12 meters upwind (45.9 μg/plate) in the same study. But, the study noted that all detections decayed to relatively low concentrations within 2 hours after application (< 2 ug/m^3; Currier et al., 1982). Airborne carbaryl degrades after reaction with hydroxyl radicals in the atmosphere (Kao, 1994). Sun et al. (2005) determined the reaction rate constant for carbaryl hydroxyl radical reactions at $3.3x10^{-11}$ cm^3/second.

Low drift concentrations were reported in a California study with concentrations up to 1.12 μg/m^3 in the air after ground spraying to control the glassy-winged sharpshooter, *Homalodisca coagulate* (Walters et al., 2003). Although below the adverse health effect concentration (51.7 μg/m^3), the study indicated that the insecticide was present in the air up to 47 hours after application (Walters et al., 2003). Shehata et al. (1984) also reported atmospheric concentrations that ranged from 0.0035 to 0.107 μg/m^3 in a Maine forest treated with carbaryl to control the spruce budworm.

In eastern France, air concentration measurements for carbaryl at remote (non-populated), rural (population = 80,000), and urban (population = 300,000) sites were on average 280, 348, and 577 pg/m^3 with highest detections at 1800, 696, and 1420 pg/m^3, respectively (Sanusi et al., 2000). The increased urban and rural carbaryl concentrations were primarily due to local agricultural use (Sanusi et al., 2000). Similar concentrations were observed in 1995 at three urban and agricultural sites along the Mississippi River (Foreman et al., 2000). However, carbaryl was detected more frequently in urban sites than agricultural sites in Mississippi and Iowa and possibly reflective of its growing domestic use (Foreman et al., 2000).

B. Water

Carbaryl is moderately soluble in water and its solubility increases with increasing temperature and amount of organic solvents. Detections of carbaryl have been found in surface waters of 42 U.S. states at low concentrations (μg/L). In many states, detections were found in both agriculture and urban environments (Table 2). Several state reported higher frequency of detections in urban than in agricultural environment. In California, detections in urban environments are less than in agricultural areas (Table 2). Carbaryl ranked 8th nationally among pesticides for outdoor home-and-garden use in 1992 (Whitmore et al., 1992), and one of four insecticides most commonly detected in urban streams in 2001 (Gilliom et al., 2007). Agricultural inputs of carbaryl to water systems have also been reported. In Florida, Wilson et al. (2006) detected carbaryl in eight of 457 samples collected from Ten Mile Creek located in an agricultural watershed at concentrations that ranged from 0.33-0.95 μg/L. Lower concentrations of carbaryl (10-100 ng/L) were detected in the Pinios River in Greece with seasonal use of the insecticide in the Thessaly agricultural area (Fytianos et al., 2006). Higher concentrations have been detected in several locations after carbaryl was used across central California to control the newly introduced glassy-winged sharpshooter pest, *Homalodisca coagulate*. For instance, 6.94 μg/L in a goldfish pond and 1737 μg/L in rain runoff in a drain were detected adjacent to where carbaryl was sprayed (Walters et al., 2003).

Groundwater detections are also reported by LaFleur (1967) who found the presence of carbaryl within two months after application to Congaree soil (well drained loamy soil on river bed) with detections continuing up to eight months. Table 2 shows that New Jersey had the highest number of carbaryl detections in groundwater across all land use types. Several other states also had groundwater detections mainly in urban and mixed-use areas.

Table 2. Detection of carbaryl in U.S. surface and ground water according to the U.S. Geological Survey [*].

State	Carbaryl			
	Type of Land use	Surface Water Detections	Ground Water Detections	Concentration Range (μg/L)
Alabama	Urban	61	1	0.002-0.422
	Agriculture	19	2	
	Mixed	41	1	
California	Urban	166	-	0.0005-5.20
	Agriculture	251	1	
	Mixed	432	1	
Alaska	Urban	20	-	0.002-0.332
Colorado	Urban	190	-	0.0005-16.5
	Agriculture	27	-	
	Mixed	126	3	
Florida	Urban	39	-	0.003-0.441
	Agriculture	21	-	
	Mixed	39	-	
Georgia	Urban	208	1	0.001-1.90
	Agriculture	20	-	
	Mixed	177	-	
Hawaii	Mixed	8	-	0.007-0.370
	Urban	9	-	
Indiana	Urban	119	-	0.001-0.460
	Agriculture	69	-	
	Mixed	62	-	
New Jersey	Urban	122	5	0.001-1.50
	Agriculture	24	5	
	Mixed	89	9	
Pennsylvania	Urban	119	-	0.001-2.41
	Agriculture	82	1	
	Mixed	82	9	
Texas	Urban	164	7	0.001-5.18
	Agriculture	13	-	
	Mixed	138	4	
Virginia	Urban	165	2	0.002-2.0
	Agriculture	14	-	
	Mixed	45	3	
Washington	Urban	46	-	0.001-33.5
	Agriculture	267	1	
	Mixed	106	2	
Wisconsin	Urban	27	-	0.002-0.267
	Agriculture	8	-	
	Mixed	40	-	

[*] U.S.G.S., 2007.

C. Soil

Organic compound sorption to soils, in general, may prevent surface and groundwater contamination and in this section the sorptive processes of carbaryl are reviewed. Carbaryl sorption to soil is rapid at 0.5 hours (Ahmad et al., 2001a) and 3 hours (Jana and Das, 1997) but persistent (from two to 16 weeks) with a $t_{½}$ of ~8 days for concentrations ranging from 1-14 mg/L (Rajagopal et al., 1984). Carbaryl has been found to adsorb more readily to acidic soil (Rajagopal et al., 1984). Both mineral and organic matter in soils has been found to contribute to carbaryl sorption. The mineral interactions are clearly reported in several recent studies. For instance, Sheng et al. (2001) found that potassium (K) saturated smectite clay (a non-ionic, expandable, hydrophilic clay) is a better sorbent for carbaryl than soil organic matter (SOM); the distribution coefficient (K_d) for carbaryl was five times greater in clay (235) than SOM rich soil (muck; 54.2). Sheng et al. (2001) estimated that K saturated clay contributes approximately 35 times more to carbaryl retention than a soil with 2% SOM. De Oliveira et al. (2005) found that its sorption is dependent on the surface charge density and is site-specific. For example, the amount of carbaryl sorbed was strongly dependent on the presence of specific exchangeable cations and followed the order of Ba ~ Cs ~ Ca > Mg ~ K > Na ~ Li. The carbonyl group in carbaryl was found to directly interact with the exchangeable cations; Mg^{2+} and Na^{+} interacted strongly with the partial negative charge of the double-bonded oxygen atom on the insecticide (De Oliveira et al., 2005). A positive correlation between carbaryl sorption with surface area, cation exchange capacity (CEC), and free Al_2O_3 content in Ultisol and Inceptisol soils was made by Jana and Das (1997). Sorption isotherms of carbaryl sorption to Indian soils followed reversible S-shaped curves which suggest multilayer adsorption on the sorbent surface (Jana and Das, 1997).

Organic matter is another contributor to sequestering carbaryl in soils. For example, carbaryl movement through soil was found to be a function of SOM content; ~52% carbaryl was leached in ten rinses from organic rich soil while it took only one rinse to leach the same amount from a sandy soil (Sharom et al., 1980). The positive contribution of SOM to carbaryl sorption is evident in Table 3 where the sorption capacity (K_f) increases with SOM content in Indian soils (Jana and Das, 1997).

Table 3. The relationship between soil organic matter (SOM) and the sorption capacity (K_f) in four different soils from India (Jana and Das, 1997).

Soil	SOM (%)	K_f (μg/g)/(μg/mL)
Ultisol 1	0.40	0.308
Inceptisol 2	1.10	1.916
Ultisol 2	1.16	2.175
Inceptisol 1	1.70	2.490

Table 4 summarizes a large data set on the sorption of carbaryl to soils from four countries (Ahmad et al., 2001a). While the table shows that organic carbon influences the sorption (K_d) of carbaryl, a positive correlation between the two was not observed by Ahmad et al. (2001a). However, in a similar study by the same group (Ahmad et al., 2001b), a positive, highly significant, correlation of organic carbon normalized sorption capacity (K_{oc}) and aromatic content of SOM was observed. Similar K_d values to those presented in Table 4 are reported elsewhere (Bondarenko and Gan, 2004) and indicate the sorption of carbaryl to soils is not very significant.

Sorption processes are predicted to be highly reversible for carbaryl since the binding is proposed to be nonspecific sorptive binding unlike chemisorption (Rajagopal et al., 1984). This, along with reported low K_d values, indicate that soils do not have a significant potential to stop carbaryl movement, with time, into water systems and other environmental fate processes (i.e., abiotic or biotic degradation) may play an important role in its dissipation.

Table 4. Distribution coefficients (K_d) for carbaryl in several soils with different organic carbon (OC) content.

Soil	OC (g/kg)	K_d	Sand:Silt:Clay (%)
Pakistan 2 [a]	2.79	0.99	22:60:18
Australian 2 [a]	3.0	0.19	92:5:3
United Kingdom 2 [a]	8.9	1.09	10:67:23
Pakistan 1 [a]	13.82	59.67	22:51:27
Australian 1 [a]	58	23.02	63:16:21
United Kingdom 1 [a]	83.8	8.80	18:39:43
California 1 [b]	-	43.4	-
California 2 [b]	-	47.7	-

[a] Ahmad et al., 2001a; [b] California 1 and 2 represent sediment from San Diego Creek and Bonita Creek in California, USA (Bondarenko and Gan, 2004).

IV. Degradation

A. Abiotic

1. Hydrolysis

Carbaryl is effectively hydrolyzed in water and undergoes 50% loss at 20ºC and pH 8 in 4 days (Rajagopal et al., 1984). Earlier studies reported similar degradation times: 6 days in flowing canal water (Osman and Belal, 1980) and one week in river water (Eichelberger and Lichtenberg, 1971). These and other investigators (Ghauch et al., 2001) showed that hydrolysis of the compound increases with elevated temperature. Hydrolytic degradation was shown to be mediated by hydroxyl radical oxidation (Fig. 2; Wang and Lemley, 2002). 1-naphthol was identified as the primary degradation product of carbaryl (Osman and Belal, 1980).

2. Photolysis

Carbaryl was photolyzed into 1,2-naphthoquione, 1,4-naphthaoquinone, 2-hydroxy-1,4-naphthoquinone, and 7-hydroxy-1,4-naphthoquinone (Brahimia and Richard, 2003). Carbaryl in water produced naphthoxyl radicals and demonstrated the hemolytic cleavage of the carbon-oxygen bonds. In oxygen rich water, however, solvated electrons could be transformed into

super-oxide anions that can recombine with radical cations or with 1-naphthoxyl radicals. Both reactions are expected to produce naphthoquinones after reduction (Brahmia and Richard, 2003).

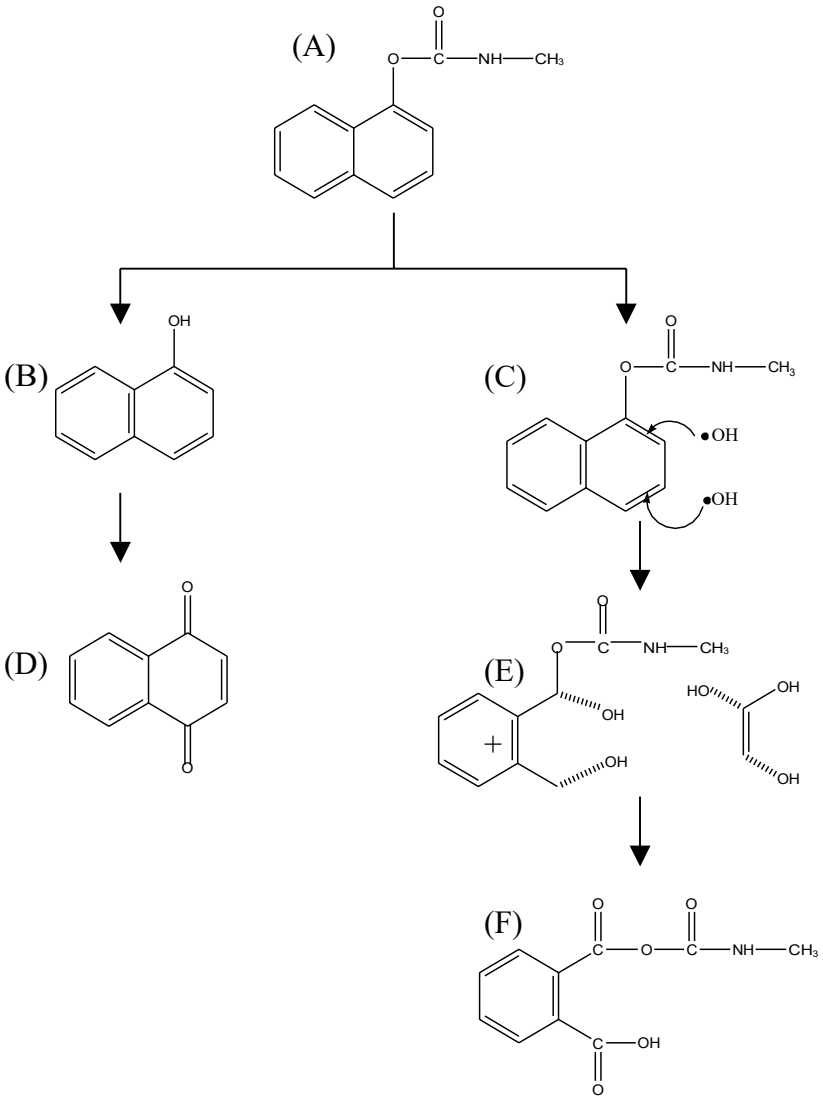

Fig. 2. The degradation pathways of carbaryl (A) by hydroxyl radical attack (C and E) showing the degradation products; 1-naphthol (B), 1,4-naphthoquinone (D), and (F) (phthalic acid-*O*-)yl *N*-methylcarbamate (Wang and Lemley, 2002).

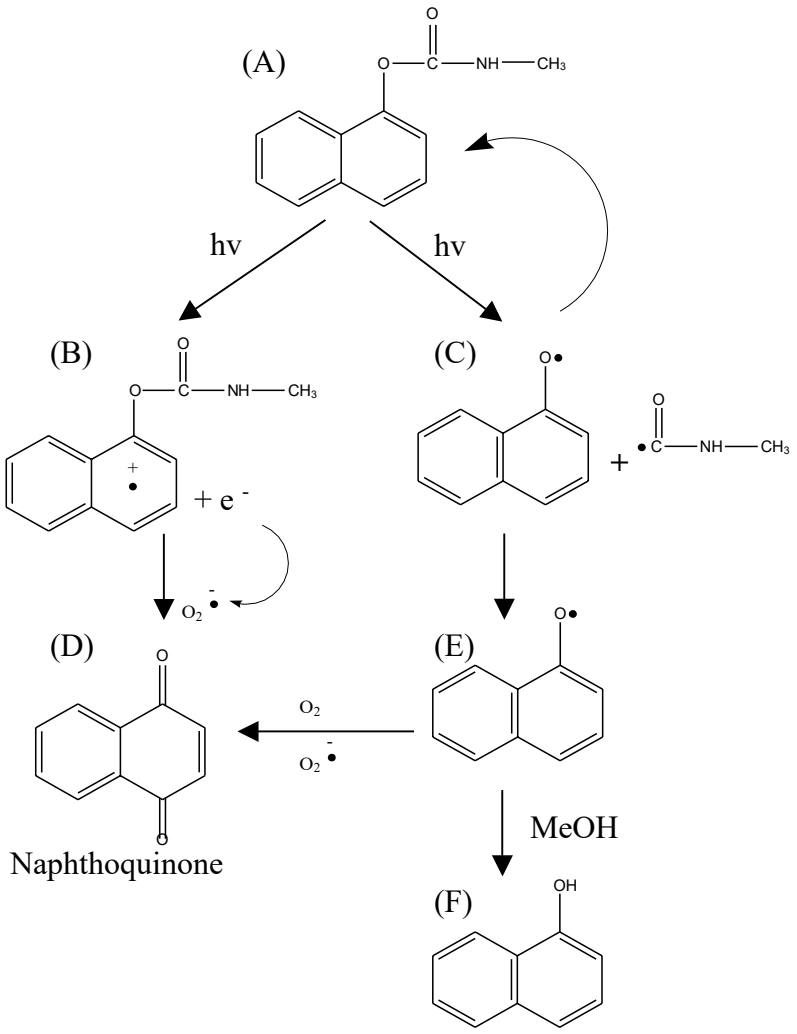

Fig. 3. Proposed (Brahmia and Richard, 2003) photolytic degradation pathway for carbaryl (A). The parent compound is distributed into radicals (B and C) via photolytic processes. 1-naphthoxyl (C) may then react with oxygen to yield naphthoquionone (D) or 1-naphthol (F). 2003). Proposed photolysis is shown (Fig. 3). Photo-conversion of carbaryl to 1-naphthol (Fig. 3 F) was also observed in organic solvents; acetonitrile and methanol.

Indirect photolysis of carbaryl has been reported by Miller and Chin (2002). They found that photo-enhanced degradation was seasonally and spatially dependent. Nitrate and dissolved organic matter (DOM) were primary constituents responsible for the formation and reaction of hydroxyl radicals with carbaryl (Miller and Chin, 2002).

B. Biotic

1. Microbial

The microbial degradation of carbaryl has been reported in several studies. For instance, ring ^{14}C-labeled carbaryl degraded at a constant rate in 120 days leaving behind 15-20% of the parent compound in the soil as monitored by the release of ^{14}C carbon-dioxide (Rodriguez and Dorough, 1977). Shorter degradation times have been observed by Menon and Gopal (2003) that carbaryl dissipated in 45 days (DT_{50} = 14.93). However, this relatively rapid degradation was attributed to high temperatures and precipitation. Still shorter DT_{50}'s have been reported that ranged from 0.15 (Wolfe et al., 1978) to several days (Tomlin, 2003). In aerobic soils the DT_{50} was 7-14 days in sandy loam and 14-28 days in clay loam soils (Tomlin, 2003). Bondarenko and Gan (2004) observed aerobic $t_{½}$ values of 1.8 and 4.9 days in soils containing 1.8 % (sand:silt:clay = 76:15:9) and 1.25 % (sand:silt:clay = 46:32:22) organic matter, respectively. First-order kinetics described the microbial degradation of carbaryl in most soils (Venkateswarlu et al., 1980). Inhibition of carbaryl can occur when ammonium nitrogen is added to the enrichment cultures (Rajagopal et al., 1983) indicating that nitrogen on the carbamate chain may provide an essential element to microbes.

Degradation has been observed to be more rapid in flooded (anaerobic) soils than aerobic soils; $t_{½}$ was 13-14 days in flooded soils while it was 23-28 days in aerobic soils (Venkateswarlu et al., 1980). Rajagopal et al. (1983) observed a DT_{50} of 10-15 days in submerged laterite and sodic soils. They also observed that degradation was faster in soils previously treated with carbaryl. Recently however, Bondarenko and Gan (2004) reported different findings. Under anaerobic conditions, carbaryl was found to be slowly degraded with $t_{½}$ values from 125-746 days depending on soil conditions, sorption capacity, and ageing of the soil with the insecticide.

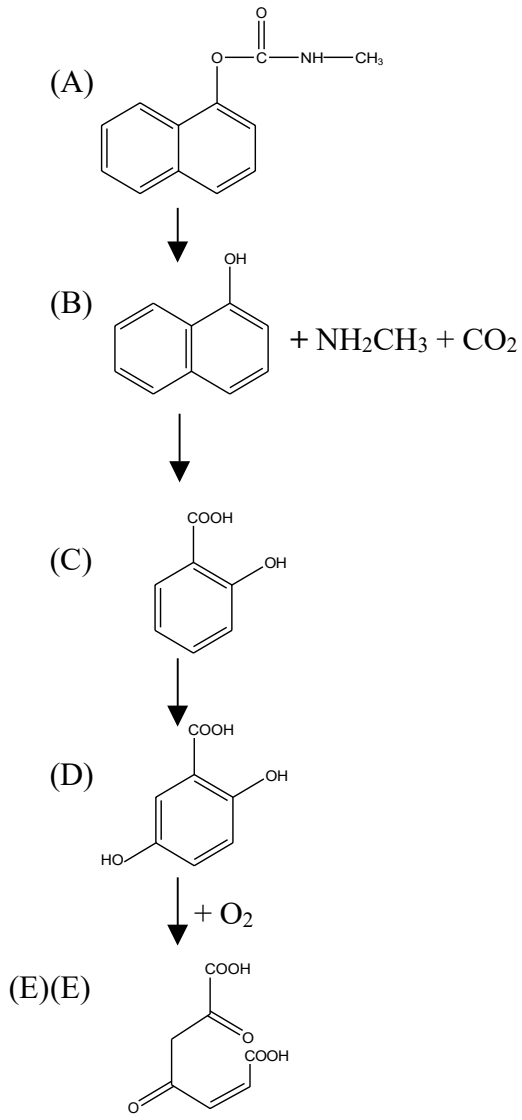

Fig. 4. Proposed degradation pathway of carbaryl by *Micrococus* sp. (Doddamani and Ninnekar, 2001). Carbaryl (A) is reduced to 1-naphthol and methylamine (B) which is then degraded to salicylic (C) and gentrisic (D) acid. The acids are then oxidized to maleylpyruvate (E).

The mechanisms of degradation have also been reported. Karinen et al. (1967) showed carbaryl ring degradation to CO_2 from 1-naphthol, its primary degradate. Thus, ring structure hydroxylation of carbaryl is the first step in microbial dissipation. Such findings are supported by Rajagopal et al. (1983) where it was noted that hydrolysis at the carbamate bond was the major pathway of degradation in flooded (anaerobic) soils (Fig. 4). The primary degradation product, 1-

naphthol, has a DT_{50} of approximately 12-14 days (Menon and Gopal, 2003) and can be further transformed to phenolic radicals which polymerize to organic matter in soils (Rajagopal et al., 1984). Complete degradation from carbaryl to maleylpyruvate is reported for an isolated *Micrococcus* species (Fig. 4) by Doddamani and Ninnekar (2001).

Other microbial strains capable of degrading carbaryl have been identified. These include bacterial species *Achromobacter*, *Pseudomonas*, *Arthrobacter*, *Xanthomonas* (Rajagopal, 1984), and *Pseudomonas cepacia* (Venkateswarlu et al., 1980). Degradation by a fungus *Penicillium implicatum* has also been demonstrated (Menon and Gopal, 2003). However, the insecticide has been shown to be inhibitory to the growth of several strains of rhizobia (Rajagopal et al., 1984).

2. Higher-order organisms

The metabolism of carbaryl has been extensively studied and evaluated for mammals. In general, the compound does not accumulate in mammalian tissue and is rapidly metabolized to non-toxic substances, particularly 1-naphthol, which are eliminated in the urine and feces (Tomlin, 2000). The main metabolic pathways in higher-order organisms are hydroxylation, hydrolysis, and expoxidation (Carpenter et al., 1961; Dorough and Casida, 1964). Hydrolysis of carbaryl by earthworms forms 1-naphthol according to Stenersen (1992). A hydrolytic degradation mechanism has been proposed by Sogorb et al. (2002). According to this pathway, carbaryl reacts with tyrosine residues on rabbit serum albumin molecules to yield 1-naphthol and carbamylated rabbit serum albumin. Water molecules than attack the carbamylated complex, releasing carbamic acid and free enzymes, the latter of which is subject to a new catalytic cycle. Carbamic acid is expected to decompose to CO_2 and methylamine (Sogorb et al., 2002). Metabolites detected in urine of human workers exposed to the carbaryl were 1-naphthyl-glucoronide and 1-naphthylsulphate (Sogorb et al., 2004). Carbaryl metabolism in human liver

microsomes and by cytochrome P450 isoforms was investigated by Tang et al. (2002). They found three major metabolites: 5-hydroxycarbaryl, 4-hydroxycarbaryl, and carbaryl methylol (Fig. 5). Interestingly, these are the same metabolites in plants (Tomlin, 2003).

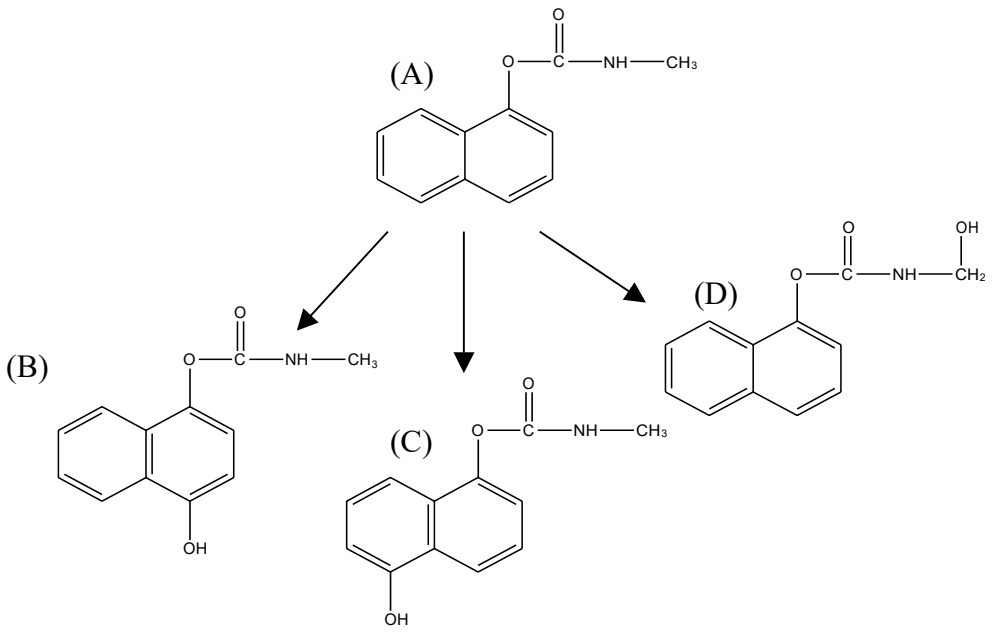

Fig. 5. The cytochrome P450-dependent metabolism of carbaryl (A) to 4-hydroxycarbaryl, 5-hydroxycarbaryl (C), and (D) carbaryl methylol (Tang et al., 2002).

Factors inhibiting enzymatic carbarylase-driven hydrolysis has also been noted. For instance, data collected by Sogorb et al. (2004) suggest that long chain fatty acids are better inhibitors of carbarylase than shorter ones. Several organic compounds can inhibit carbarylase as well. For example, chlorpyrifos inhibits carbaryl metabolism (Tang et al., 2002) and paraoxon inhibits carbalylase by 44% (Sogorb et al., 2004).

V. Toxicity

Carbaryl is a highly effective insecticide for controlling insect pests. For example, it is used to control several animal ectoparasites, specifically the cattle tick *Boophilus microplus*. This tick is endemic to Mexico, having been eradicated from the U.S. in 1961 according to Li et al.

(2005). Several strains of *B. microplus* were highly susceptible to carbaryl; LC_{50} ranged from 0.0025 to 0.0031% (Li et al., 2005). Carbaryl is highly toxic to the bee at 1 μg (LD_{50}) topical dose (Tomlin, 2003).

Although carbamate pesticides do not persist in the environment, there may still be short-term cumulative effects on the reproduction of aquatic organisms. For instance, Tripathi and Singh (2004) found that doses of 2, 5, and 8 mg/L carbaryl altered the biochemical parameters in nervous, hepatopancreatic, and ovotesticular tissues of the snail, *Lymnaea acuminate*. Specifically, glycogen, pyruvate, total protein, and nucleic acid levels were reduced after 96 hours of exposure to carbaryl while lactate and free amino acid levels increased (Tripathi and Singh, 2004). Carbaryl can also affect embryo development. For example, Tripathi and Singh (2004) reported that the number of eggs for the freshwater snail, *Lymnaea acuminate*, were reduced by 49% at 2 mg/L while no eggs were laid at 5 and 8 mg/L. The rate of neonatal survival was also reduced significantly by 53% after exposure of hatchling for 28-day at 2 mg/L. In a similar study Todd and Van Leeuwan (2002) found that the average mortality of zebrafish eggs (*Danio rerio*) was reduced (~20%) after low-level exposures (<0.05 mg/L). Although the insecticide did not directly kill embryos, it had a significant effect on embryo size.

When zebrafish were exposed to 0.017 mg/L of carbaryl, they developed more slowly and hatched later compared to the controls. Delayed hatching exposes zebrafish embryos to predations. The toxicity results of carbaryl to several aquatic animals are summarized in Table 5. Note that carbaryl is toxic to the water flea, shrimp, and freshwater snail at ppb (μg/L) levels while to fish at ppm (mg/L). The results suggest that the insecticide should not be used in bodies of water or in fields adjoining those bodies, particularly in the rainy season.

Table 5. The aquatic animal toxicology of carbaryl.

Aquatic organism	Test	Concentration (mg/L unless noted)
Juvenile trout [a]	96 h LC_{50}	4.27-6.18
Toad larvae [a]	96 h LC_{50}	17.68-34.77
Juvenile trout [a]	IC_{50}	19 µg/L
Toad larvae [a]	IC_{50}	7.580
Rainbow trout [b]	96 h LC_{50}	1.3
Sheephead minnow [b]	96 h LC_{50}	2.2
Bluegill sunfish [b]	96 h LC_{50}	10
Mysid shrimp [b]	96 h LC_{50}	5.7 µg/L
Eastern oyster [b]	48 h LC_{50}	2.7
Shrimp larvae [c]	96 h LC_{50}	30 µg/L
Common carp [d]	96 h LC_{50}	7.85
Freshwater snail [e]	24 h LC_{50}	20.05
Freshwater snail [e]	96 h LC_{50}	14.19
Water flea (*B. longirostris*) [f]	24 h LC_{50}	8.6 µg/L
Water flea (*B. fatalis*) [f]	24 h LC_{50}	4.1 µg/L
Water flea predator (*L. kindtii*) [f]	24 h LC_{50}	3.6 µg/L

[a] Ferrari et al., 2004; [b] Tomlin, 2003; [c] Reyes et al., 2002; [d] De Mel and Pathiratne, 2005, [e] Tripathi and Singh, 2001; [f] Sakamoto et al., 2005.

Rats and dogs tolerate carbaryl at 200 and 400 mg/kg, respectively (Carpenter et al., 1961). Table 6 summarizers LD_{50} data of carbaryl to several birds having a greater tolerance to the compound compared to other animals such as rats and dogs.

Table 6. The oral LD_{50} of carbaryl to birds.

Bird	Concentration (mg/kg)
Mallard duck [a]	>2179
Pheasant [a]	>2000
Japanese quail [a]	2230
Pigeon [a]	1000-3000

[a] Tomlin, 2003.

VI. Summary

Carbaryl is an agricultural and garden insecticide that controls a broad spectrum of insects. It is moderately soluble and does not volatilize readily nor easily vaporize. The compound is susceptible to drift after spray application, unstable under alkaline conditions, and easily hydrolyzed. It has been detected in water at µg/L concentrations but degradation of

carbaryl in this environmental medium is relatively rapid with 1-naphthol identified as the major degradation product. Indirect and direct photolysis of carbaryl produces different naphthaquinones as well as some hydroxyl substituted naphthaquinones.

The pesticide's sorption to soil is kinetically fast and both the mineral and organic fractions of soil contribute to its relatively low sorption. Sorption to soil minerals was strongly dependent on the presence of specific exchangeable cations and increased with the soil organic matter aromaticity and age. Microbes in soils (bacteria and fungi) are capable of degrading carbaryl and the process is more rapid in anoxic than aerobic systems and with increased temperature and moisture.

In mammals, the compound does not accumulate and is rapidly metabolized to non-toxic substances which are eliminated in the urine and feces. Several studies have shown that a cumulative effect may exist with respect to the reproduction of aquatic organisms. Therefore, its application near water bodies must be carefully evaluated prior to its use.

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ENVIRONMENTAL FATE OF CARBARYL

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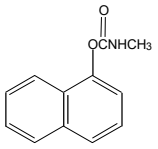
The document reviews the environmental fate and environmental effect of carbaryl (1-naphthyl-N-methyl carbamate). Carbaryl is one of the most frequently used carbamate insecticides and widely used for the control of a variety of pests on fruit, vegetables, forage, cotton and many other crops, as well as on poultry, livestock and pets (Mathew et al., 1995). It is available as wettable powders, pellets, granules, dusts, suspensions and even solutions (U.S. EPA, 1988).

Physical and chemical properties

Common Name	Carbaryl
Chemical Name	1- naphthalenylmethylcarbamate
Trade Names	Arilat, Arilate, Arylam, Carbacine, Karbaryl, Menaphtam, Sevin, Vioxan
CAS Registry No.	63-25-2
Structural Formula	
Empirical Formula	$C_{12}H_{11}NO_2$
Molecular Weight	201
Water Solubility	113 ppm at 22^0C (R&P, 1988) 40 ppm at 30^0C (Kidd and James, 1991)
Organic Solvents Solubility at 25^0C(R&P, 1988)	Methanol: 7960 ppm

	Hexane: 214 ppm
	Methylene Chloride: 242,600 ppm
Vapor pressure	1.17 x 10^{-6} mmHg @25^0C (R&P, 1988)
	4.1 x 10^{-2} mPa @ 23.5^0C(Kidd and James, 1991)
Octanol/water partition coefficient	
	70.8 ($\log K_{ow}$ = 1.85) at 25^0C(R&P, 1988)
	$\log K_{ow}$ = 2.36 (Kenaga and Goring, 1980)
Henry's law constant (R&P, 1988)	2.7425 x 10^{-9} atm m^3g.mol^{-1} at 25^0C
Environmental Fate	
Hydrolysis Half-Life	>1500 days (pH = 5) (Wolfe et al., 1978)
	12.1 days (pH = 7) (Carpenter, 1990)
	3.2 hours (pH = 9) (Carpenter, 1990)
Soil Adsorption Coefficient (K_{oc})	251 (Sablijic, 1995)
	100-600 (WHO, 1994a)
Photolysis Half-Life (Water, Artificial Light, pH = 5)	21 days (Das, 1990a)
Photolysis Half-Life(Soil, Artificial Light)	41 days (Das, 1990b)
Aerobic Soil Half-Life (WHO, 1994b)	4-17 days (sandy loam soil)
	21-27 days (clay loam soil)
Anaerobic Half-life	78 days (Miller, 1993a)
Field Dissipation Half-life	0.76 – 10.9 days (Norris, 1991)
Toxicity and Ecological Effects (Kidd and James, 1987)**:**	
Rat (oral, acute)	LD_{50} 850 mg/kg (males)
	LD_{50} 500 mg/kg (females)
Rabbit (oral, acute)	LD_{50} 710 mg/kg
Rat (inhalation, acute)	LC_{50} >206.1 mg/l air
Mallard Duck (oral, acute)	LD_{50} >2179 mg/kg
Pheasants (oral, acute)	LD_{50} >2000 mg/kg
Japanese Quail (oral, acute)	LD_{50} 2230 mg/kg
Pigeons (oral, acute)	LD_{50} 1000-3000 mg/kg

Channel Catfish (96 hrs)	LC_{50} 15.8 ppm (Tucker, 1970)
Asellus (96 hrs)	LC_{50} 280 ppb (Tucker, 1970)
Daphnia Magna (48 hrs)	LC_{50} 18.6 ppb (Li and Yang, 2000)
Daphnia Magna(48 hrs)	EC_{50} 0.26 ppb (Rawash et al. 1975)
Daphnia Magna (Surprenant, 1985)	21-day MATC 1.5-3.3 ppb
	21-day NOEC 6.0 ppb
Fathead Minnow (96 hrs)	LC_{50} 5.29 – 10.4 ppm (Brooke, 1984)
Brine Shrimp (Barahona and Sanchez-Fortun, 1999)	
(24 hrs)	
(48 hrs)	LC_{50} 5.90 ppm
(72 hrs)	LC_{50} 0.35 ppm
Rainbow Trout (96 hrs)	LC_{50} 4.38 ppm
Bluegill Sunfish (96 hrs)	LC_{50} 6.76 ppm
Goldfish (96 hrs)	LC_{50} 13.2 ppm
Sow Bug (Asellus Brevicaudus) (96 hrs)	280 ppb (Johnson and Finley, 1980)
Glass Shrimp (Palaemonetes) (96hrs)	LC_{50} 5.6 ppb (Johnson and Finley, 1980)
(Kadiakensis)	LC_{50} 120 ppb (Chaiyarach et al., 1975)
Blue Crab (48 hrs)	LC_{50} 320 ppb (Mayer, 1987)
Brown Shrimp (48 hrs)	LC_{50} 1.5 ppb (Mayer, 1987)
Grass Shrimp (48 hrs)	LC_{50} 28 ppb (Mayer, 1987)
Mysid shrimp (96 hrs)	LC_{50} > 7.7 ppb (Nimmo et al., 1981)
Honey Bee	LD_{50} 1.54 – 26.5 µg a.i/bee (Union Carbide, 1983)

Mode of Action

Carbaryl is a member of the widely used carbamate pesticides. Like most carbamates, carbaryl acts as an inhibitor to cholinesterase, one of many important enzymes in the nervous systems of humans, vertebrates and insects (Extoxnet, 2000). A specific cholinesterase enzyme, acetylcholinesterase (AChE), plays an important role in breaking down the acetylcholine (Ach), which is the synaptic mediator of nerve impulses in the

nervous systems of mammals and insects (WHO, 1994d). The presence of cholinesterase inhibiting pesticides, such as carbaryl, prevents AChE from breaking down acetylcholine and results in high concentration of Ach in the nervous system. As a result, the continuous stimulation of the muscle leads to uncontrolled, rapid movement of some muscles, paralysis, convulsions and even death.

Environmental Fate

Air: Carbaryl has a low vapor pressure, 1.17×10^{-6} mmHg, and is not readily volatilized into the air. A low Henry's law constant, 2.74×10^{-9} atm $m^3 g.mol^{-1}$, suggests that carbaryl has low potential to volatilize from aqueous solution (Lyman et al., 1982). It might be found in the atmosphere associated with air-borne particulates or as spray drift but should not be over a large area. If existed in the air, carbaryl tends to react with hydroxyl radical in the ambient atmosphere (Kao, 1994). Carbaryl in air was monitored after being applied to a large area of forest in Maine for the control of spruce budworm, and the concentrations ranged from 0.0035 to 0.107 $\mu g/m^3$ (Shehata et al., 1984).

Water: Hydrolysis is the primary degradation pathway for carbaryl at pH 7 or above. The compound degrades rapidly at pH 7 and 9 at 25^0C, with half-lives of approximately 10~17 days and 3 hours, respectively (Aly&El-Dib, 1971; Carpenter, 1990). In acidic water, carbaryl is rather stable with a half-life of more than 1500 days at 27^0C (Wolfe et al., 1978). The identified degradation products are 1-napthol, methylamine and CO_2 (Aly and ElDib, 1971; Larkin and Day, 1986). In natural water, carbaryl is expected to degrade faster due to the presence of microorganisms. The half-lives of carbaryl in steams, rivers and brooks as a result of forest spraying are 25, 28 and 23 hours, respectively (Stanley et al., 1980).

The aqueous photolysis of carbaryl was determined to be 21 days in sterile distilled water under artificial sunlight at a concentration of 10.1 ppm and pH 5 (Das, Y.T., 1990a). The intensity of artificial light is comparable to that of the natural sunlight, at 510.5 and 548.8 watts/m^2, respectively. Other reported aqueous photolysis half-lives are much shorter

than that obtained from the sterile water. Wolfe et al. (1978) has reported that photolysis half-life of carbaryl is 6.6 days, and Zepp et al. (1977) as 50 hours near water surface. The aqueous photolysis rates increase as intensity of sunlight increased; therefore, the rate of hydrolysis is much faster in summer than that in winter. Wolfe et al. (1976) has calculated aqueous photolysis half-lives of carbaryl in surface water (in < 10 cm water) at latitude 40 degrees North in different seasons: 64 hours in spring, 52 hours in summer, 102 hours in fall and 200 hours in winter. The major photolysis product is 1-naphthol, which will further photooxidize to 2-hydroxy-1,4-naphtho-quinone in basic condition (Wauchope and Haque, 1973).

The soil sorption coefficients (K_{oc} = 100 ~ 600), octanol/water partition coefficients ($\log K_{ow}$ = 1.85 – 2.36) and water solubility indicate that carbaryl moderately binds to soils and sediments. Thus, suspended particulates or mud in natural water may remove some carbaryl from the aqueous phase. Karinen et al. (1967) reported that 50% of initial carbaryl disappeared from estuarine water after 38 days at 8^0C in the absence of mud; in the presence of mud, 90% of initial applied carbaryl was withdrawn from the water after 10 days at the same temperature due to significant removal of carbaryl by mud.

Carbaryl may enter marine system resulting from the control of oyster pests and predators (Haven et al., 1966). Carbaryl is believed to be more persistence in seawater than in freshwater (WHO, 1994c). Armbrust et al. (1991) reported that hydrolysis half-lives of carbaryl in filtered and sterilized seawater at pH 7.9 and 8.2 at 24^0C were 24 and 23 hours, respectively, and the major degradation product was 1-naphthol. Naphthol was not degraded in dark sterile seawater but was undetected within 96 hours in raw seawater. If exposed to artificial sunlight, carbaryl had a half-life of 5 hours and naphthol was completely degraded in 2 hours.

Soil: Overall, carbaryl is not persistent in soil. It can be degraded through hydrolysis, photolysis as well as by microorganisms. The photodegradation of carbaryl was investigated on soil under artificial sunlight for a total of 30 days (Das, 1990b). In this

case, carbaryl was applied on 1-mm soil layers at a concentration of 9.8 ppm. The estimated half-life was approximately 41 days with no findings of major metabolites.

Microbes play a significant role in the degradation of carbaryl in soil. Quite a few bacteria can use carbaryl as their sole source of carbon and nitrogen. Chapalamadugu et al. (1991) revealed that two Pseudomonas spp, which were isolated from soil, can metabolize either carbaryl or 1-naphthol. A bacterial consortium, constructed by two isolates, is able to completely catabolize carbaryl to CO_2 within36 hours. The mechanism of the metabolism of 1-naphthol by Pseudomonas spp is proposed as via salicyclic acid (Larkin and Day, 1986).

In aerobic soil, carbaryl was quickly degraded with an approximate half-life of 4 days (Miller, 1993a). A significant amount of CO_2 was produced, ranging from 0.1% at day 1 to 59.7% at day 14. Another major degrade is 1-naphthol. Carbaryl degrades more slowly in anaerobic aquatic soil with an estimated half-life of 72 days (Miller, 1993b). 1-naphthol is the major degradate with minor compounds of 1,4-naphthoquinone, 5-hydroxy-1-naphthyl methylcarbamate and 1-naphthyl-(hydroxymethyl) carbamate. None of these minor degradates was accounted for more than 2.5% of total applied dose. CO_2 was generated slowly, ranging from none at day 3 to 4% at the day of 94. At day 126, CO_2 reached the maximum of 23.6%.

Murthy et al. (1989) studied the metabolism of ^{14}C-carbaryl and 1-naphthol in moist and flooded soils over a 28-day period. More CO_2 was generated from carbaryl treated moist soil than from flooded soil. Most radio-activities existed as soil bound materials and only less than 1 percent of parent was present in extractable radiocarbon. The major degradation was 5-hydroxyl carbaryl in moist soil and 4- and 5-hydroxyl carbaryl in flooded soil.

The adsorption coefficient values (K_{oc}) of carbaryl range from 100 to 600 (WHO, 1994a; Jana and Das, 1997), indicating carbaryl moderately binds to soil. Sorption experiments were implemented on two types of soils, Red Bay (AB) and Astatula (AS), which were

further separated into two layers, topsoil (0-30 cm) and subsoil (31-60 cm) (Nkedi-Kizza and Brown, 1998). The properties of individual soil are: AB top (pH 6.3,OM 15.2%), AB sub (pH 5.3, OM 3.9%), AS top (pH 5.6, OM 8.0%) and AS sub (pH 4.8, OM 2%). The sorption coefficient values (K_{oc}) of carbaryl on soils are: 338, 144, 590 and 671 mg/kg on AB topsoil, AB subsoil, AS topsoil and AS subsoil, respectively. The half-lives of carbaryl on the four soils ranged from 8 to 18 days. Given a same soil, carbaryl degraded much faster in topsoil than in subsoil.

Terrestrial field dissipation studies were conducted at two locations, one in California and one in North Carolina (Norris, 1991). Data showed that most residues remain in the first 0-0.15 meters of soil, with only one finding in the layer of 0.3 –0.45 meter. The dissipation half-lives of carbaryl were estimated as from 0.76 to 10.9 days.

Biota: The efficacy of carbaryl for the control of pests is attributed to its ability to inhibit acetylcholinesterase (Ache) in the nervous systems (Barabona and Sanchez-Fortun, 1999). Given the same mode of action, carbaryl also poses risks to other non-target animals, including human beings. Carbaryl can penetrate the skin, mucous membranes, respiratory tract and gastrointestinal tract of mammals. However, it can be rapidly metabolized by various animals, and excreted especially in the urine as glucuronides or sulfates (Dorough and Casida, 1964; Fukuto, 1972). The following metabolites have also been identified: 1-naphtyl N-hydroxymethylcarbamate, 4-hydroxy-1-naphthyl-N-methylcarbamate, 5-hydroxyl-1-naphthyl-N-methyl-carbamate and 5,6-dihydroxy-1-naphthylmethylcarbamate.

Carbaryl is relatively safe to mammals although it can temporarily inhibit AchE. Rats given a single oral dose of 560 mg/kg body weight showed a decrease of 42% erythrocyte- and 30% brain-ChE activity within 5 minutes (Carpenter et al., 1961). However, the activity recovered to normal level after 24 hours.

Carbaryl and its major degradate, 1-naphthol, are toxic to some ecologically beneficial soil microorganisms such as *Chlorella vulgaris*, *Nostoc linckia* and *Synechococcus*

elongates (Megharaj et al., 1990). Obulakondaiah et al. (1993) reported that carbaryl and 1- naphthol resulted in toxicity at concentrations of 50-100 ppm and 25-100 ppm, respectively. In this case, 1-naphthol was found to be more toxic than its parent compound since it inhibits nitrogen cycling mediated by tested microorganisms. Under other circumstances, carbaryl presented more toxic effect on different microbes than 1-naphthol (Megharaj et al., 1990).

Carbaryl is considered moderately to highly toxic to fish with LC_{50} values ranging from 4 ppm to 13 ppm (Beyers, et al., 1994; McKim, 1987; Sinha et al., 1991). The chemical is especially toxic to the aquatic invertebrate *Daphnia magna* with LC_{50} values at 48 hours less than 18.6 ppb (Li and Yang, 2000). Weis et al. (1974) reported that 0.1-ppm carbaryl water solution is able to disrupt the schooling habit for juvenile *Menida medidia*. 1-naphthol is believed to be the major factor instead of the parent compound. However, schooling behavior was recovered within 3 days.

Carbaryl is slightly or practically non-toxic to birds, with LD_{50} for young mallard ducks, young pheasants and pigeons of >2179, 2000, 1000-3000 mg/kg, respectively. The effect of low concentration carbaryl (1.68 kg/ha) on nontarget birds, mammals and insects have been investigated in western North Dakota (George et al., 1992). No evidence was found to conclude that carbaryl depressed brain AChE in birds or small mammals collected from the treated area after 2, 10, 21 days or 1 year.

Carbaryl is highly toxic to honey bees, with LD_{50} values ranging from 1.5 to 26.5 ug a.i. per bee (Union Carbide, 1983). Study revealed that adjusting the application time and formulation of carbaryl could significantly reduce the toxicity of carbaryl to honey bee. To minimize the death of honey bee, applications could be made during early morning or late evening when bees are not actively foraging.

The metabolism of carbaryl in plants is similar to that in animals. Several water-soluble metabolites were recovered from crops treated with ^{14}C-carbaryl, including 5,6-hydroxy-5,6-dihydrocarbaryl, N-hydroxymethylcarbaryl, 4- and 5-hydroxycarbaryl and 1-naphthol

(Kuhr, 1967; Kuhr and Casida, 1967; Kuhr, 1970). Both hydrolytic and oxidative reactions contributed equally to the metabolism of carbaryl.

The persistence of carbaryl on plants have been investigated by several research groups (Choudhary,et. al,1988; Galhotra, et. al., 1985; Iwata, et. al.,1979, Rao and Ramasubbaiah, 1988). Sevin 80W were applied on mature orange and lemon trees at the rate of 11.5 lb a.i. $(1200\ gal)^{-1}\ acre^{-1}$ in Orange County and Riverside County California, respectively (Iwata, et al., 1979). After 5 days, foliar residues for orange were 5.6 ug/cm^2 and lemon 2.4 ug/cm^2. The residues after 60 days were 0.36 and 0.41 ug/cm^2 on orange and lemon, respectively. The half-lives of carbaryl on oranges and lemons were reported as 14 and 22 days, respectively. In other experiments, the dissipation half-lives of carbaryl were 1.80-1.94 days in Sesamum and less than 1.25 days in tomato (Choudhary,et. al, 1988 and Galhotra, et. al., 1985). Galhotra (1985) reported the carbaryl residues in potato foliage and tuber, 64-94 days after application at the rates of 1 – 5 kg a.i. /ha, were below detectable level (0.03 – 0.10 ppm).

The side effects of carbaryl on the growth of plants have been well documented (Murthy and Raghu, 1990; Jones et al, 1991). Undesirable thinning of apples have been observed after paclobutrazol and carbaryl were applied to apple trees within an interval of seven days in a period over 20-30 days after full bloom Carbaryl with a concentration of 2.5 ppm in clay and sandy loam soils had no effect on the growth of barley. However, higher concentration of carbaryl, 25 and 100 ppm, demonstrated the inhibitory effects (Murthy and Raghu, 1990). The phototoxic effects of carbaryl only lasted for a few days after the application of carbaryl in soil.

Conclusion

Carbaryl is a contact and respiratory poison, functioning as a reversible inhibitor of cholinesterase (ChE) activity. In general carbaryl is slightly toxic to mammals, moderately to highly toxic to aquatic organisms and honey bees. Carbaryl is degraded rapidly in plants.

Carbaryl does not readily volatize into the atmosphere and it is unlikely to volatize from water to air. Carbaryl moderately binds to soil and has potential to leach to groundwater (Guo, 2000). It is not persistent in soil since it can be hydrolyzed, photodegraded, oxidized as well as microbial-degraded.

In alkaline or neutral water, hydrolysis is the major degradation route for carbaryl, with half-lives ranging from few hours to few days. It is also subject to microbial degradation in natural water. The photolysis plays a role in the degradation process, significantly reducing degradation half-life of carbaryl. The major degradation product is 1-naphthol.

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