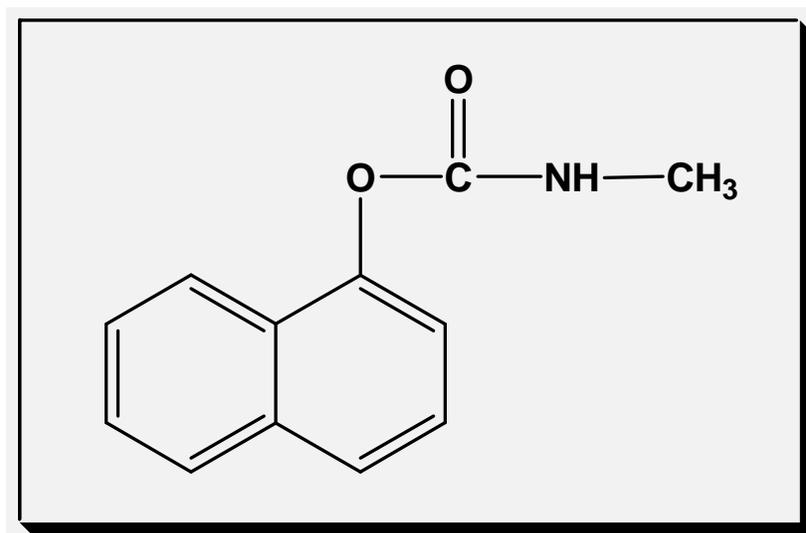


**Carbaryl  
(1-naphthyl methylcarbamate)**

OCCUPATIONAL AND BYSTANDER  
RISK CHARACTERIZATION DOCUMENT



**Medical Toxicology Branch  
Department of Pesticide Regulation  
California Environmental Protection Agency**

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DRAFT---DO NOT CITE

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## ABBREVIATIONS

<b>AADD</b>	annual average daily dosage
<b>ACh</b>	acetylcholine
<b>AChE</b>	acetylcholinesterase
<b>ACGIH</b>	American Conference of Governmental Industrial Hygienists
<b>ALH</b>	amplitude of lateral head velocity
<b>ATSDR</b>	Agency for Toxic Substances and Disease Registry
<b>BCF</b>	beat cross frequency
<b>CASA</b>	computer aided semen analysis
<b>ChE</b>	cholinesterase
<b>DPR</b>	Department of Pesticide Regulation (California EPA)
<b>EEG</b>	electroencephalogram
<b>FIFRA</b>	Federal Insecticide, Fungicide and Rodenticide Act
<b>FOB</b>	functional observational battery
<b>FQPA</b>	Food Quality Protection Act
<b>gd</b>	gestation day
<b>GSD</b>	geometric standard deviation
<b>HPLC</b>	high pressure liquid chromatography
<b>IREDD</b>	Interim Reregistration Eligibility Document
<b>LADD</b>	lifetime average daily dosage
<b>LC/MS</b>	liquid chromatography / mass spectrometry
<b>LD<sub>50</sub></b>	dose required to kill 50% of exposed animals
<b>LED</b>	lower bound on the effective dose
<b>LED<sub>10</sub></b>	lower bound on the dose required to achieve a 10% benchmark response
<b>LOEL</b>	lowest observed effect level
<b>MMAD</b>	mass median aerodynamic diameter
<b>MOE</b>	margin of exposure
<b>MTD</b>	maximum tolerated dose
<b>NOEL</b>	no observed effect level
<b>OSHA</b>	Occupational Safety and Health Administration
<b>PCNA</b>	proliferating nuclear cell antigen
<b>ppd</b>	<i>post partum</i> day
<b>PEL</b>	permissible exposure limit
<b>PHED</b>	pesticide handlers exposure database
<b>RBC</b>	red blood cell
<b>RED</b>	Reregistration Eligibility Document (US EPA)
<b>SCCNFP</b>	Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers
<b>SADD</b>	seasonal average daily dosage
<b>SD</b>	Sprague-Dawley
<b>STADD</b>	short-term absorbed daily dosage
<b>TDM</b>	tail distributed moment
<b>TLC</b>	thin layer chromatography
<b>TRR</b>	total radioactive residues
<b>TSCA</b>	Toxic Substances Control Act
<b>TWA</b>	time weighted average
<b>USEPA</b>	United States Environmental Protection Agency

<b>VAP</b>	mathematically smoothed velocity
<b>VCL</b>	curvilinear velocity
<b>VSL</b>	straight line velocity
<b>WH&amp;S</b>	Worker Health and Safety Branch (DPR)

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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's 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.

Carbaryl is used on 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, flowables, granules, soluble concentrates and suspension concentrates.

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 November 2011 there were 24 formulations containing carbaryl registered in California, with concentrations ranging between 0.126% and 99.45%. Several of these formulations also contain metaldehyde. DPR released a dietary risk characterization document on carbaryl in 2010--  
<http://www.cdpr.ca.gov/docs/risk/rcd/carbaryl.pdf>.

### **Illness and injury reports**

One hundred illnesses in which carbaryl played a definite, probable or possible role were reported to DPR between 1992 and 2008. These occurred in 73 separate incidents. Seventy two of the cases occurred in fieldworkers and handlers combined. The 44 fieldworker cases were dominated by systemic adverse effects (21 cases) and effects in skin (14 cases). The 28 handler cases also showed systemic and skin effects (8 and 2 cases, respectively), as well as eye (7 cases), "skin-systemic" (4 cases) and "respiratory-systemic" (4 cases) effects. Finally, there were 28 "other" (*i.e.*, miscellaneous) cases, including 6 cases resulting from exposures to carbaryl dust from torn packaging and 7 ingestion cases, of which 5 were intentional. The "other" cases were dominated by "respiratory-systemic" effects (11 cases), skin effects (5 cases), "skin-systemic" effects (5 cases) and "eye-respiratory-systemic" effects (4 cases).

### **Environmental fate**

Despite its 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 phenomenon. Hydrolysis, which is favored at pH 7 and above, and photolysis both occur under aqueous conditions. The presence of microorganisms enhances hydrolytic degradation. 1-naphthol, methylamine and CO<sub>2</sub> are hydrolytic breakdown products. The major photolysis product is 1-naphthol, which photooxidizes to 2-hydroxy-1,4-naphtho-quinone under alkaline 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, breakdown is enhanced under *acidic* conditions. Carbaryl has a moderate ability to bind to soil, a process 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 enhanced by organic matter. Carbaryl and 1-naphthol are toxic to some beneficial soil-dwelling microorganisms, though 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 is relatively non-toxic to birds. Metabolism of carbaryl in plants is similar to that in animal systems (see below).

### **Pharmacokinetics**

Orally administered carbaryl was excreted primarily through the urine in rats during the first 24 hours, though substantial residues appear in feces and in exhaled air as CO<sub>2</sub> (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 of 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 followed the kinetics of [naphthyl-1-<sup>14</sup>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 (guinea pig, sheep) were generally similar to those in the rat. However, there were problems with the latter studies, including the fact that they were conducted during the 1960s, tested few animals and left large fractions of the administered dose unanalyzed. A possible exception to the rat kinetic 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 mostly 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 rat urinary metabolites -- 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 detected in the urine of a single dosed monkey. The toxicologic significance of these species differences was not clear. Humans 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, another study showed that intentionally dosed humans excreted only 25-30% of the carbaryl in the urine by 24 hours, suggesting that the fate of a significant fraction of the dose was unknown.

### **Hazard identification**

The acute toxicity of carbaryl results largely 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.

**Acute oral toxicity.** Rats exhibited acute oral LD<sub>50</sub>s between 233 and 840 mg/kg. Mice were between 108 and 650 mg/kg. 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) 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 measurement on gestation day 9, three days after the commencement of dosing. 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. Another plausible interpretation of the data (but with somewhat weaker experimental support) resulted in an alternative critical acute LED<sub>10</sub> of **0.25 mg/kg**. This 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 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. Furthermore, USEPA established a LED<sub>10</sub> of 1.1 mg/kg based on inhibition of brain cholinesterase activity in postnatal day 11 rats following a single gavage exposure to carbaryl.

**Subchronic oral toxicity.** The risk from subchronic oral exposure to carbaryl was evaluated using the critical chronic oral value of 0.5 mg/kg/day.

**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- $\sigma$  / 11.2- $\text{♀}$  mg/kg/day and 33.8- $\sigma$  / 34.4- $\text{♀}$  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 activities 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<sub>10</sub> value.

The Hill algorithm for continuous data generated the best curve fit for 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 was observed throughout the study, even at the high dose of ~34 mg/kg/day. The critical chronic LED<sub>10</sub> for brain cholinesterase inhibition in females using the Hill algorithm was **0.5 mg/kg/day** (ED<sub>10</sub> = 1.7 mg/kg/day). This value was used to evaluate the non-oncogenic risks from annual (*i.e.*, chronic) exposure to carbaryl.

**Acute, subchronic and chronic dermal toxicity.** The risk from acute, subchronic and chronic dermal exposure to carbaryl was evaluated using a dermal NOEL of 20 mg/kg/day. The LOEL of 50 mg/kg/day was based on statistically significant inhibition of brain cholinesterase activity after 4 weeks of daily exposure (6-7 hr/day, 5 days/wk) in a repeat dose dermal toxicity study. Brain cholinesterase activities measured on day 26 of that study 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 ( $p < 0.05$ ). This subchronic study was the only one available to assess dermal systemic toxicity.

**Acute inhalation toxicity.** The risk from acute inhalation exposure to carbaryl was evaluated using the critical acute oral value of 1 mg/kg. Support for this determination was forthcoming from an acute inhalation toxicity study showing an LED<sub>10</sub> (ED<sub>10</sub>) of 9.81 (14.15) mg/m<sup>3</sup>, which was equivalent to an internal dose of 1.18 (1.70) mg/kg, based on inhibition of brain cholinesterase activity at 10 mg/m<sup>3</sup>. However, the oral NOEL was considered primary because it was based not only on enzyme inhibition, but on overt cholinergic signs (see above).

**Subchronic and chronic inhalation toxicity.** As neither a subchronic nor a chronic inhalation study was available, the critical chronic oral LED<sub>10</sub> of 0.5 mg/kg/day from the 1-yr dog dietary study was used.

**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  $\alpha$ -naphthol (1-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 period 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 from a role in carbaryl-induced cancers in mice. For this reason, and for reasons of appropriate curve-fitting, the multistage cancer model was used to define carbaryl's tumor potency using the incidence curve for hemangiosarcomas / hemangiomas in "at risk" male mice. The top dose was excluded because it exceeded the maximum tolerated dose. The human

potency values were  $1.01 \times 10^{-2} \text{ mg/kg/day}^{-1}$  (the 95% upper bound estimate) and  $5.03 \times 10^{-3} \text{ mg/kg/day}^{-1}$  (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 definitively link carbaryl exposure and human seminal defects, though the data were suggestive of an increase in oligospermia (defined as a sperm count  $<20 \times 10^6/\text{ml}$ ) 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, there was a positive correspondence between urinary 1-naphthol levels and various indicators of sperm toxicity in 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 in 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). 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 with total fetal deaths (one each at 6.25, 25 and 50 mg/kg/day); (3) decreased pup weight gains in all of the 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”.

### **Risk calculations**

The potential for non-oncogenic health effects resulting from exposure to carbaryl was expressed as the Margin of Exposure (MOE), *i.e.*, the critical NOEL or  $\text{LED}_{10}$  divided by the estimated exposure. A MOE of  $>100$  was generally considered to be protective of human health when the relevant adverse effects were observed in animal studies, as was the case in the present assessment.

**Occupational, bystander and ambient exposure and risk.** Estimates of exposure to carbaryl resulting from various occupational, bystander and ambient scenarios were developed by the Worker Health and Safety Branch (WH&S) of DPR. Assumptions regarding application rates, acres treated/day, dermal and inhalation absorption and default body weight were detailed in the accompanying exposure assessment document. When necessary, surrogate exposure

estimates from the Pesticide Handlers Exposure Database were used. MOEs and cancer risk values are summarized as follows.

Occupational handler and occupational reentry risk (dermal and inhalation exposure)

- Short-term exposure: many MOEs less than 100, with several less than 1
- Seasonal exposure: many MOEs less than 100
- Annual exposure: several MOEs less than 100
- Lifetime exposure / oncogenic risk: generally in excess of  $10^{-6}$ , reaching as high as  $4.24 \times 10^{-3}$  for airblast mixer / loaders (handlers) and  $1.02 \times 10^{-2}$  for citrus pruners (reentry workers)

Residential handler and residential reentry risk (dermal and inhalation exposure)

- Short-term exposure: dermal MOEs less than 100 for backpack mixer / loader / applicators and residential reentry onto carbaryl-treated turf (adults and toddlers), inhalation MOEs less than 100 for push-type spreader loader / applicator

Toddler risk - hand-to-mouth, object-to-mouth and soil ingestion behaviors

- Short-term exposure: all MOEs equal to or greater than 100

Swimmer risk (dermal and oral exposure)

- Short-term, seasonal and annual exposures: all MOEs substantially greater than 100

Bystander risk (inhalation exposure)

- 1-hr exposure: MOEs less than 100 for infants (heavy activity)
- Short-term exposure: inhalation MOEs less than 100 for 1-hr risk (infants, heavy activity), short-term risk (infants and adults)
- Oncogenic risk:  $1.88 \times 10^{-6}$

**Dietary exposure and risk.** Risk from dietary exposure to carbaryl was evaluated in a previous DPR document, as noted above (<http://www.cdpr.ca.gov/docs/risk/rcd/carbaryl.pdf>). The contribution of dietary exposure to aggregate risk is summarized in the following section.

**Aggregate risk.** There were several occupational handler exposure scenarios that exhibited individual MOEs of greater than 100 by the dermal and inhalation routes, but less than 100 either when the two routes were aggregated using the hazard index approach or when a third route, dietary exposure was included. For the latter, the aggregate risk calculations assumed acute and chronic dietary MOEs of 228 and 1973, respectively, for the working population (see DPR's dietary risk assessment for details). MOEs dipped below 100 for four handler scenarios and one adult bystander scenario, for which the individual contributing MOEs were greater than 100 in each case: (1) short-term groundboom applicators (dermal + inhalation + dietary); (2) short-term high-acre broadcast spreader applicators (dermal + inhalation + dietary); (3) seasonal airblast citrus applicators (dermal + inhalation); (4) annual high-pressure handwand mixer / loader / applicators (dermal + inhalation); and (5) adult bystanders to agricultural applications (inhalation + dietary). Many other aggregate MOEs were also below 100, but in each of those cases at least one of the individual contributing MOEs was already below 100.

**Toxicity of carbaryl metabolites**

**1-Naphthol.** Human exposure to 1-naphthol occurs through the metabolism of carbaryl or

naphthalene. Exposure is also plausible through (1) the use of 1-naphthol 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<sub>50</sub> was 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 a dose-related rise in white blood cell counts in 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 for teratogenic or other adverse effects in the developing embryo / fetus after dermal exposure up to 10 mg/kg/day every 3 days throughout gestation in the rat. 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 absence 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.

**Methylamine.** Methylamine is produced upon hydrolytic breakdown of carbaryl, which occurs under alkaline conditions. It is known as an irritant in eyes, nose and throat upon brief exposures to 20 - 100 ppm. Severe methylamine exposure may precipitate pulmonary edema. The oral LD<sub>50</sub> 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.

Neither 1-naphthol nor methylamine was factored into the risk calculations for carbaryl.

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

Carbaryl has been listed as a developmental toxin and a male reproductive toxin under Proposition 65 since August 2009, and as a carcinogen since February 2010.

## 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. The chemical is also registered in California as a molluscicide and acaricide, 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, flowables, granules, soluble concentrates and suspension concentrates (Crop Protection Handbook, 2009).

As a member of the carbamate class of pesticides, the action of carbaryl is based on its ability to inhibit acetylcholinesterase (AChE) in the nervous systems of target species. 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 directed toward 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 in the present risk evaluation. 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 (IREL), 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 IREL also cited possible impacts on non-target organisms and endangered species that may require mitigation. U.S. EPA initiated a registration review for carbaryl, which is required every 15 years under FQPA (the Food Quality Protection Act), on September 22, 2010 (U.S. EPA, 2010).

Requests for cancellation of all carbaryl uses were submitted by the Natural Resources Defence Council and the Washington Toxics Coalition after publication of the USEPA IREL. However, 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, those registrants choosing 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 to "meet the safety standard set forth in section 408(b)(2) of the FFDCA" (USEPA, 2007a and 2007c).

Carbaryl has been listed as a developmental toxin and a male reproductive toxin under Proposition 65, the Safe Drinking Water and Toxic Enforcement Act of 1986, since August 7, 2009, and as a carcinogen since February 5, 2010. The Proposition 65 list, which is annually revised and now includes about 800 substances, "requires businesses to notify Californians about significant amounts of chemicals in the products they purchase, in their homes or workplaces, or that are released into the environment. By providing this information, Proposition 65 enables Californians to make informed decisions about protecting themselves from exposure to these chemicals. Proposition 65 also prohibits California businesses from knowingly discharging significant amounts of listed chemicals into sources of drinking water" (<http://oehha.ca.gov/prop65/background/p65plain.html>).

Carbaryl also ranks at #270 (of 275) on ATSDR's 2011 priority list of "substances that are most commonly found at facilities on the National Priorities List (NPL) and which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure at these NPL sites. This substance priority list is revised and published on a 2-year basis, with a yearly informal review and revision. (No list was published in 2009 while ATSDR transitioned to a new agency science database.) Each substance on the list is a candidate to become the subject of a toxicological profile prepared by

ATSDR. The listing algorithm prioritizes substances based on frequency of occurrence at NPL sites, toxicity, and potential for human exposure to the substances found at NPL sites." (<http://www.atsdr.cdc.gov/SPL/index.html>).

DPR issued a dietary risk characterization document on carbaryl in 2010 (DPR, 2010: <http://www.cdpr.ca.gov/docs/risk/rcd/carbaryl.pdf>). Two critical acute values were established in that document. Use of the first, more clearly supported NOEL value of 1 mg/kg (based on cholinergic signs and brain cholinesterase inhibition at 10 mg/kg in a rat study (Robinson and Broxup, 1997)) resulted in MOEs less than the health protective standard of 100 in three of 17 subpopulations analyzed: all infants, non-nursing infants <1 yr, and children 1-2 yr. Use of the second, somewhat less clearly supported LED<sub>10</sub> value of 0.25 mg/kg (based on slight hypotonic gait at 1 mg/kg in the same study) resulted in MOEs less than 100 for 16 of 17 subpopulations and for the U.S. population as a whole. Using a critical chronic LED<sub>10</sub> value of 0.5 mg/kg/day (based on brain cholinesterase inhibition in a dog study (Hamada, 1987)) resulted in no annual MOEs less than 100. However, use of an oncogenic potency value of 10<sup>-3</sup> mg/kg/day<sup>-1</sup> (based on induction of hemangiosarcomas and hemangiomas in mice (Hamada, 1993b)), resulted in oncogenic risk values greater than the health protective standard of 10<sup>-6</sup> in all adult populations analyzed.

### **C. TECHNICAL AND PRODUCT FORMULATIONS**

Carbaryl is registered in California as an insecticide, acaricide 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 in November 2011, there were 24 carbaryl-containing products actively registered in California. The carbaryl concentrations in these products range between 0.126% and 99.45%. Several of these formulations contain 2% metaldehyde in addition to carbaryl. Registered products include aqueous dispersions, baits, dusts, flowables, granules, soluble concentrates and suspension concentrates.

#### **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 2009 (Table II-1). In 2009 about 131,000 pounds of carbaryl were applied commercially, compared to 821,000 pounds in 1994. Commodities receiving the highest amounts of carbaryl in pounds applied in 2009 were processing tomatoes (39,851 lb), oranges (22,221 lb), apples (10,308 lb), cherries (5958 lb) and olives (5938 lb). In 2008 those commodities were processing tomatoes (27,180 lb), oranges (22,478 lb), apples (9298 lb) and pistachios (8522 lb). In 2007 they 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 under non-agricultural scenarios (Table II-1). By this calculation, an average of 61% ( $\pm 15\%$ ) of the total carbaryl sold was applied under agricultural conditions over the 1994-2009 period, with about 39% 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. Total pounds of carbaryl sold compared with pounds applied agriculturally in California, 1994-2009

Year	Pounds sold <sup>a</sup>	Pounds applied <sup>a</sup>	Agricultural fraction(%) <sup>b</sup>
1994	1,264,283	820,787	65
1995	1,242,400	835,811	67
1996	834,427	810,162	97
1997	1,142,675	754,659	66
1998	506,764	427,546	84
1999	639,600	388,144	61
2000	563,605	364,060	65
2001	412,635	286,199	68
2002	421,528	256,098	61
2003	329,782	205,102	62
2004	388,235	240,135	62
2005	412,955	190,633	46
2006	411,711	156,938	38
2007	323,069	142,010	44
2008	289,524	126,076	44
2009	277,240	130,981	47

<sup>a</sup> 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](http://www.cdpr.ca.gov/dprdatabase.htm)

<sup>b</sup> "Agricultural fraction", expressed as a percent, represent the pounds applied divided by the pounds sold for a given year. This is a rough estimate of the fraction that is applied commercially, mostly under agricultural conditions.

## **E. ILLNESS REPORTS**

One hundred illness cases in which carbaryl played a definite, probable or possible role were reported to DPR between 1992 and 2008 (DPR, 2011). These occurred in 73 separate episodes. Seventy two of the cases occurred in fieldworkers and handlers combined. The 44 fieldworker cases were dominated by systemic adverse effects (21 cases) and effects in skin (14 cases). The 28 handler cases also showed systemic and skin effects (8 and 2 cases, respectively), as well as eye (7 cases), “skin-systemic” (4 cases) and “respiratory-systemic” (4 cases) effects. Finally, there were 28 “other” (*i.e.*, miscellaneous) cases, including 6 cases resulting from exposures to carbaryl dust from torn packaging and 7 ingestion cases, of which 5 were intentional. The “other” cases were dominated by “respiratory-systemic” effects (11 cases), skin effects (5 cases), “skin-systemic” effects (5 cases) and “eye-respiratory-systemic” effects (4 cases).

A detailed summary of the above reports, including charts and tables, appears in the accompanying Human Exposure Assessment Document for Carbaryl (DPR, 2012).

## 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 at <http://www.cdpr.ca.gov/docs/emon/pubs/envfate.htm>.)

<b>Chemical names</b>	1-naphthyl N-methylcarbamate; 1-naphthenol methylcarbamate; methyl carbamic acid 1-naphthyl ester
<b>CAS registry number</b>	63-25-2
<b>Molecular weight</b>	201.2
<b>Molecular formula</b>	C <sub>12</sub> H <sub>11</sub> NO <sub>2</sub>
<b>Physical state</b>	White crystalline solid (Union Carbide in support of registration 169-058)
<b>Melting point</b>	142°C
<b>Density</b>	1.23 @ 20°C
<b>Solubility in water</b>	113 ppm @ 22°C, 40 ppm @ 30°C <sup>a</sup>
<b>Solubility in organic solvents</b>	methanol: 7960 ppm; hexane: 214 ppm; methylene chloride: 214,600 ppm
<b>Vapor pressure</b>	1.17 x 10 <sup>-6</sup> mm Hg 25°C
<b>Log octanol-water partition coefficient (log K<sub>ow</sub>)</b>	1.85 - 2.36
<b>Henry's Law constant</b>	2.74x10 <sup>-9</sup> atm m <sup>3</sup> g/mol at 25°C
<b>Hydrolysis half-lives</b>	>1500 days @ pH 5; 12.1 days @ pH 7; 3.2 hr @ pH 9
<b>Aqueous photolysis half-life</b>	21 days (artificial light; pH 5)
<b>Soil photolysis half-life</b>	41 days (artificial light)
<b>Aerobic soil half-life</b>	4 - 17 days (sandy loam); 21-27 days (clay loam)
<b>Anaerobic degradation half-life</b>	78 days
<b>Field dissipation half-life</b>	0.76 - 10.9 days
<b>Adsorption coefficient (K<sub>oc</sub>)</b>	261

<sup>a</sup> 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 can be found at <http://www.cdpr.ca.gov/docs/emon/pubs/envfate.htm>

**Air.** Carbaryl has a low vapor pressure ( $1.17 \times 10^{-6}$  mm Hg 25°C) and low Henry's Law constant ( $2.74 \times 10^{-9}$  atm m<sup>3</sup> g/mol at 25°C), both of which decrease the tendency toward volatilization. In spite of this, 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<sub>2</sub> were identified as hydrolytic breakdown products. The presence of microorganisms is expected to enhance hydrolytic degradation. The photolysis 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 alkaline 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 adsorption 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

The pharmacokinetic handling of carbaryl has been examined primarily in rats, but also in mice, guinea pigs, monkeys, dogs and humans, through a series of studies conducted over several decades. 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<sub>2</sub> (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, 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.

These studies, which utilized oral, intravenous and dermal exposure routes, are summarized in the following section.

##### 2. Pharmacokinetics in laboratory animals and humans

Struble *et al.* (1994) studied the absorption, distribution, metabolism and excretion of <sup>14</sup>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<sub>2</sub> 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-1a and 1b show 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 glutathione 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.<sup>1</sup>

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<sup>1</sup> 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 <sup>14</sup>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 <sup>a</sup>	nd	0.04± 0.042	55.5± 8.17	49.6± 8.54	51.5± 16.56	19.3± 3.73	nd <sup>a</sup>	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 <sup>b</sup>	3.50± 2.091 <sup>b</sup>	2.49± 1.071 <sup>b</sup>	5.81± 2.555 <sup>b</sup>
0-24 <sup>d</sup>	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 <sup>c</sup>	0.46 <sup>c</sup>	0.48 <sup>c</sup>	1.01 <sup>c</sup>
<b>Total</b>	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 <sup>14</sup>C-carbaryl, iv; Group B, 1 mg/mg <sup>14</sup>C-carbaryl, oral gavage; Group C, 1 mg/mg <sup>14</sup>C-carbaryl oral gavage after 14 days of 1 mg/mg/day unlabeled carbaryl, oral gavage; Group D, 50 mg/mg <sup>14</sup>C-carbaryl oral gavage.

<sup>a</sup> Not determined. For the cage rinse/wash/wipe determinations, the value represents the percent retrieved at the latter time point only.

<sup>b</sup> Cage rinse only.

<sup>c</sup> Cage wash + cage wipe (combined for simplicity; no standard deviation).

<sup>d</sup> 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 <sup>14</sup>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 <sup>a</sup>	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 <sup>a</sup>	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 <sup>b</sup>	9.04±4.288 <sup>b</sup>	9.56±2.292 <sup>b</sup>	6.46±2.763 <sup>b</sup>
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 <sup>c</sup>	0.65 <sup>c</sup>	0.44 <sup>c</sup>	0.49 <sup>c</sup>
<b>Total</b>	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 <sup>14</sup>C-carbaryl, iv; Group B, 1 mg/mg <sup>14</sup>C-carbaryl, oral gavage; Group C, 1 mg/mg <sup>14</sup>C-carbaryl oral gavage after 14 days of 1 mg/mg/day unlabeled carbaryl, oral gavage; Group D, 50 mg/mg <sup>14</sup>C-carbaryl oral gavage.

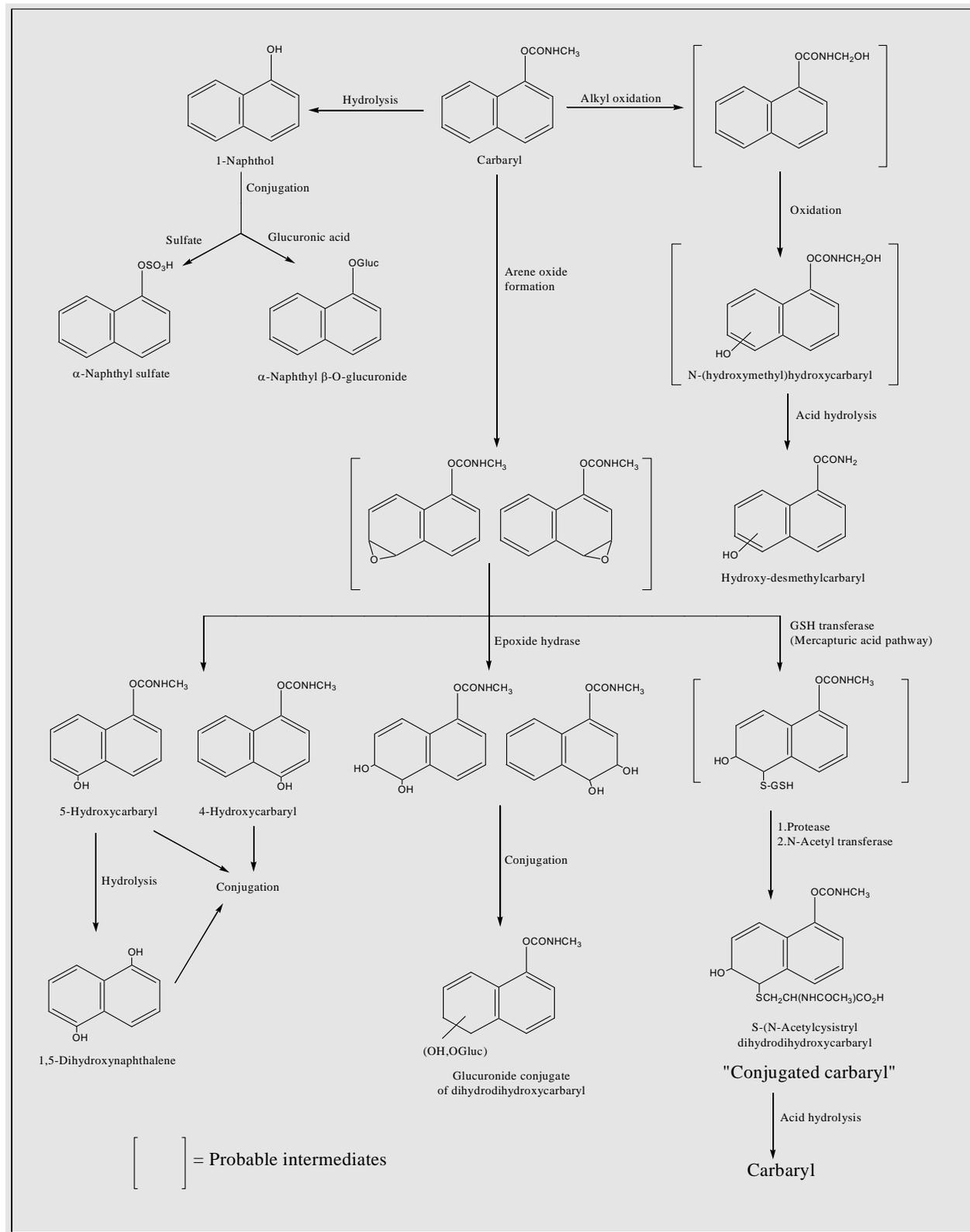
<sup>a</sup> Not determined. For the cage rinse/wash/wipe determinations, the value represents the percent retrieved at the latter time point only.

<sup>b</sup> Cage rinse only.

<sup>c</sup> Cage wash + cage wipe (combined for simplicity; no standard deviation).

<sup>d</sup> 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 <sup>14</sup>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 <sup>14</sup>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\*\*  $\mu\text{mol/g}$  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 ( $\alpha$ -naphthyl- $\beta$ -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<sub>1</sub> 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 <sup>14</sup>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)  $\alpha$ -naphthyl sulfate and (4)  $\alpha$ -naphthyl  $\beta$ -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 linkage, 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-<sup>14</sup>C]-carbaryl. Four animals per time point were euthanized at 0.25, 0.5, 1, 3 and 5 hours after the second oral dose. 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<sup>2</sup>. 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-naphtol 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-pentoxoresorufin 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.* (1965, 1967, 1968) in several species. Knaak's rat data largely support Struble (1994), though it

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<sup>2</sup>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.

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-<sup>14</sup>C, carbaryl-methyl-<sup>14</sup>C or carbaryl-carbonyl-<sup>14</sup>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<sub>2</sub> after 4 days was, for carbaryl-naphthyl-<sup>14</sup>C: 72% / 10% / 0%; for carbaryl-methyl-<sup>14</sup>C: 69% / 7% / 11%; for carbaryl-carbonyl-<sup>14</sup>C: 47% / 8% / 32%. As might be expected, carbaryl-naphthyl-<sup>14</sup>C was not detected as <sup>14</sup>C O<sub>2</sub>, while carbaryl-methyl-<sup>14</sup>C and carbaryl-carbonyl-<sup>14</sup>C produced CO<sub>2</sub> at 11% and 32% of the dose, respectively. Two to 3% of the methyl-<sup>14</sup>C dose was recovered in the intestinal tract, carcasses and remaining organs (neither naphthyl-<sup>14</sup>C nor carbonyl-<sup>14</sup>C residues were detected in tissues). Recovery data for guinea pigs were not presented.

Urinary metabolites of carbaryl-naphthyl-<sup>14</sup>C, carbaryl-methyl-<sup>14</sup>C and carbaryl-carbonyl-<sup>14</sup>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 <sup>3</sup>, 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 <sup>14</sup>C and the most prominent identifiable metabolite of carbaryl-methyl-<sup>14</sup>C in guinea pig urine at 30.1% of the recovered <sup>14</sup>C), 4-(methylcarbamoyloxy)-1-naphthyl glucuronide, 1-naphthyl glucuronide (the most prominent metabolite of carbaryl-naphthyl-<sup>14</sup>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-<sup>14</sup>C in the presence of a hydrogen donor (NADPH<sub>2</sub>) and uridine diphosphoglucuronide (UDPGH) formed a spectrum of metabolites. These included unidentified water-soluble neutrals, 4-(methylcarbamoyloxy)-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 exposed to carbaryl dust in a packaging operation at a Union Carbide plant (though the number

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<sup>3</sup> The 21% discrepancy for the carbaryl-methyl-<sup>14</sup>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.

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-naphthol conjugated with glucuronic and sulfuric acids." <sup>4</sup> In addition, the study confirmed the ability of humans to hydrolyze (decarbamylylate) 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-<sup>14</sup>C or carbaryl-methyl-<sup>14</sup>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 <sup>14</sup>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 intact carbamates 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 monkey excreted three intact carbamates, including compounds D (16-18%), F (31-38%) and H

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<sup>4</sup> 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.

(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-<sup>14</sup>C and carbaryl-methyl-<sup>14</sup>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-<sup>14</sup>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-<sup>14</sup>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-<sup>14</sup>C in the feces as in the urine presents a quantitative difference from the rat, which excretes less than 10% of the carbaryl-naphthyl-<sup>14</sup>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 <sup>14</sup>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 <sup>14</sup>C-carbofuran or <sup>14</sup>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<sub>50</sub> 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 ("weak effect") or 10 mg/kg ("strong effect"), based on cholinergic effects in rats at those dose in a developmental neurotoxicity study (Robinson and Broxup, 1997; summarized in section III.H. - Developmental Neurotoxicity). Benchmark dose analysis was used to arrive at an oral LED<sub>10</sub> of 0.25 mg/kg for the weak effect. The oral NOEL for the strong effect was 1 mg/kg. The critical LOEL for inhalation toxicity was 10 mg/m<sup>3</sup>, 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<sub>10</sub> of 9.81 (14.15) mg/m<sup>3</sup>, equivalent to an internal dose of 1.18 (1.70) mg/kg assuming a default breathing rate of 0.96 m<sup>3</sup>/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<sup>3</sup> 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, tinnitus 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<sub>50</sub>). 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<sub>CO2</sub>, 54 mm Hg P<sub>O2</sub>, 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<sup>5</sup> arm movements;
- (7) Day 6: proximal right leg movements, CSF contained 2200 RBC/mm<sup>3</sup>, 20 WBC/mm<sup>3</sup>, 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.

### **3. Laboratory animal studies**

#### **a. LD<sub>50</sub>, LC<sub>50</sub> and primary eye and skin irritation**

LD<sub>50</sub>, LC<sub>50</sub> and primary irritation data for carbaryl and for various end-product formulations

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<sup>5</sup> Athetosis: "a derangement marked by ceaseless occurrence of slow, sinuous, writhing movements, especially severe in the hands, and performed involuntarily" (Dorland's Illustrated Medical Dictionary, 26<sup>th</sup> Edition, 1985; W.B. Saunders Company; p. 134).

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 <sub>50</sub> or LC <sub>50</sub> | References |
|----------------------------------------|-------------------|--------------------------------------|------------|
| <b>Oral LD<sub>50</sub></b>            |                   |                                      |            |
| 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          |
| <b>Intraperitoneal LD<sub>50</sub></b> |                   |                                      |            |
| Rat, M-adult                           | n/a               | 64 mg/kg                             | l          |
| Rat, M-weanling (23 days)              | n/a               | 48 mg/kg                             | l          |
| <b>Dermal LD<sub>50</sub></b>          |                   |                                      |            |
| Rat, M/F                               | III               | >2000 - >5000 mg/kg                  | d          |
| Rabbit, M/F                            | III               | >2000 mg/kg                          | b,f        |
| <b>Inhalation LC<sub>50</sub></b>      |                   |                                      |            |
| Rat, M/F - 4 hours                     | III               | 0.873 mg/L                           | g          |
| Rat, M/F - 4 hours                     | III               | 2.50 mg/L                            | h          |
| <b>Eye irritation</b>                  |                   |                                      |            |
| Rabbit                                 | IV                | n/a                                  | b          |
| <b>Dermal irritation</b>               |                   |                                      |            |
| Rabbit                                 | IV                | n/a                                  | b          |
| <b>Dermal sensitization</b>            |                   |                                      |            |
| Guinea pig                             | negative          | n/a                                  | j,k        |

<sup>a</sup> Mellon Inst. (1957)

<sup>b</sup> Union Carbide (1983a-d)

<sup>c</sup> Union Carbide (1985)

<sup>d</sup> Cranmer (1986)

<sup>e</sup> Larson (1987d)

<sup>f</sup> Larson (1987a) - As the test article, Carbaryl 90DF, was "slightly moistened to make pasty", it is assumed that the moistening agent was water.

<sup>g</sup> Holbert (1989)

<sup>h</sup> Dudek (1985)

<sup>i</sup> Rybakova (1966)

<sup>j</sup> Larson (1987c)

<sup>k</sup> USEPA (2002a)

<sup>l</sup> Brodeur and DuBois (1963)

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

| Species                                                   | Tox. Categ. | LD <sub>50</sub> or LC <sub>50</sub> | References |
|-----------------------------------------------------------|-------------|--------------------------------------|------------|
| <b>Oral LD<sub>50</sub></b>                               |             |                                      |            |
| 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          |

<sup>a</sup> Myers and Homan (1978)

<sup>b</sup> Mellon Inst. (1957)

<sup>c</sup> Biosearch, Inc. (1980)

<sup>d</sup> Weatherhostz (1982)

<sup>e</sup> Myers (1983a)

<sup>f</sup> Myers (1983b)

<sup>g</sup> Myers (1985)

<sup>h</sup> Field (1980)

<sup>i</sup> Fukuda (1983)

<sup>j</sup> Duke (1982)

<sup>k</sup> Kuhn (1991a)

<sup>l</sup> Kuhn (1991b)

<sup>m</sup> Kuhn (1991c)

<sup>n</sup> Kuhn (1991d)

<sup>o</sup> Kuhn (1991e)

<sup>p</sup> Mitchell (1991)

<sup>q</sup> Kuhn (1992a)

<sup>r</sup> Kuhn (1992b)

<sup>s</sup> Myers (1987)

<sup>t</sup> Kuhn (1991f)

### b. Full acute toxicity studies

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<sub>10</sub> (ED<sub>10</sub>) 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<sub>10</sub> (ED<sub>10</sub>) 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<sub>10</sub> 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<sub>10</sub> and LED<sub>10</sub> values following a single gavage dose in male Long-Evans rats (Moser, 2007)

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

Abbreviation: nd, not determined

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

<sup>a</sup> LED<sub>10</sub> and ED<sub>10</sub> values, which are expressed as mg/kg, were calculated by the study author using an exponential algorithm.

<sup>b</sup> Brain cholinesterase activities are expressed both in units of μm ACh hydrolyzed / min / mg protein and in percent of concurrent controls.

<sup>c</sup> RBC cholinesterase activities are expressed both in units of μm ACh hydrolyzed / min / ml RBCs and in percent of concurrent controls.

<sup>d</sup> Motor activities are expressed as total counts per 20-minute test period.

<sup>e</sup> 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 to 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 sign 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



Beyrouly (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<sub>50</sub> 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, Beyrouly (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:  $1.9 \pm 1.4$ ,  $0.8 \pm 1.0^*$ ,  $0.8 \pm 0.7^*$ ,  $0.0 \pm 0.0^{***}$ ;  $*p < 0.05$ ;  $***p < 0.001$ ). 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 ( $^{\circ}\text{C}$ ):  $38.0 \pm 0.33$ ,  $37.3 \pm 0.99^*$ ,  $34.9 \pm 0.53^{**}$  and  $34.3 \pm 0.78^{**}$  ( $*p < 0.05$ ;  $**p < 0.01$ ). 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.



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 (exophthalmus, 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.



Weinberg (2008) administered aerosolized carbaryl technical (99.8% purity) through nose-only devices to two separate cohorts of CrI: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<sup>3</sup> (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<sup>3</sup>, 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<sup>3</sup>, 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 (Table III-5; 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<sup>3</sup> (1.2 mg/kg for the 3-hr exposure using the default breathing rate of 0.96 m<sup>3</sup>/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 unrestricted) resulted in LED<sub>10</sub> (ED<sub>10</sub>) values of 9.81 (14.15) mg/m<sup>3</sup>, equivalent to an internal dose of 1.18 (1.70) mg/kg (Appendix V).

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

Table III-5. RBC and brain cholinesterase activities after a 3-hr acute inhalation exposure to carbaryl in rats (Weinberg, 2008)

Cohort 1:

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

\*\*p<0.01

Cohort 2:

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

\*\*p<0.01



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  $\gamma$  (theta) wave frequency, which was statistically increased over controls at both doses ( $p < 0.01$ ) and the  $\beta_2$  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 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 and EEG characteristics 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  $\mu\text{M/ml}$ , 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  $\mu\text{M/ml}$ ), 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 not 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 margins in those samples taken within an hour of test article removal on days 5 ( $\sigma$  &  $\varphi$ ) and 12 ( $\sigma$  @ 100 mg/kg/day only,  $\varphi$  @ 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-6). 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 exposure or multiple exposures, 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<sup>2</sup> (assuming a body surface area for a 200 g rat of 325 cm<sup>2</sup> - Harkness and Wagner, 1983), based on the atonia noted at 100 mg/kg/day.

This study was considered to be supplemental.

Table III-6. 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
<b>RBC, Umol/L - Pre-daily dose assays</b>								
<b>Day -4</b>	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%
<b>Day 1</b>	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%
<b>Day 8</b>	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%
<b>Day 15</b>	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%
<b>Day 22</b>	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%
<b>RBC, Umol/L - Post-daily dose assays</b>								
<b>Day 5</b>	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%
<b>Day 12</b>	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%
<b>Day 19</b>	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%
<b>Day 26</b>	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%
<b>Brain, Umol/mg - Post-daily dose assays</b>								
<b>Day 26</b>	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 ( $\sigma$ : 2/90, 3/80, 3/80, 8/90), limited use of hind limbs ( $\sigma$ : 0/90, 2/80, 0/80, 8/90), alopecia-front limbs ( $\varphi$ : 5/90, 8/80, 8/80, 21/90), alopecia-front feet ( $\varphi$ : 2/90, 4/80, 9/80, 20/90), alopecia-multiple sites ( $\varphi$ : 0/90, 0/80, 2/80, 5/90), urine stains ( $\sigma$ : 3/90, 7/80, 2/80, 21/90;  $\varphi$ : 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-7a.

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-7b.

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-7c 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<sup>6</sup>. 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<sup>7</sup>” (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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<sup>6</sup>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, 26<sup>th</sup> Edition, 1985; W.B. Saunders Company; p. 475)

<sup>7</sup> Lipofuscin: “any one of a class of fatty pigments formed by the solution of a pigment in fat” (Dorland’s Illustrated Medical Dictionary, 26<sup>th</sup> 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 maximum tolerated dose (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-7a. Body weights and food consumption in Sprague-Dawley rats exposed over a 2-yr period to dietary carbaryl (Hamada, 1993a)

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

\* p<0.05

<sup>a</sup> 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-7b. Cholinesterase activities for Sprague-Dawley rats exposed to dietary carbaryl for 2 years (Hamada, 1993a)

Time	Carbaryl dose, males (ppm) <sup>a</sup>				Carbaryl dose, females (ppm) <sup>a</sup>			
	0	250	1500	7500	0	250	1500	7500
<b>Week -1</b>								
plasma	2.8 <sup>b</sup>	n/a <sup>c</sup>	n/a	n/a	4.8	n/a	n/a	n/a
RBC	5.6				5.6			
brain	68.1				69.6			
<b>Week 26</b>								
plasma	2.2	2.1 (5) <sup>d</sup>	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
<b>Week 52</b>								
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)
<b>Week 78</b>								
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
<b>Week 104</b>								
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

<sup>a</sup> 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.

<sup>b</sup> Plasma and RBC ChE activities expressed as  $\mu\text{mol/ml}$ ; brain ChE activity expressed as  $\mu\text{mol/g}$ .

<sup>c</sup> n/a, not applicable

<sup>d</sup> Numbers in parentheses represent the percentage of inhibition compared to concurrent controls.

Table III-7c. Neoplastic and non-neoplastic changes in Sprague-Dawley rats exposed over a 2-yr period to dietary carbaryl (Hamada, 1993a)

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

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

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

<sup>\*</sup>, <sup>\*\*</sup>, <sup>\*\*\*</sup>: p<0.05, 0.01, 0.001 (Fisher Exact Test) - tests performed by risk assessor.

<sup>+</sup>, <sup>++</sup>, <sup>+++</sup>: p<0.05, 0.01, 0.001 (Cochran-Armitage Trend Test) - tests performed by risk assessor.

<sup>a</sup> 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.

<sup>b</sup> T, terminal sacrifice; U, unscheduled deaths

<sup>c</sup> B, benign; M, malignant

<sup>d</sup> ic, intracytoplasmic; n/a, not applicable

<sup>e</sup> 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).

<sup>f</sup> Sum of "at risk" urinary bladder transitional cell papillomas and transitional cell carcinomas.

<sup>g</sup> 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.

<sup>h</sup> 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.

<sup>i</sup> 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.

<sup>j</sup> 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.

<sup>k</sup> 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.

<sup>l</sup> 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 animals in that group (and in some of the males). Interestingly, this parameter was noted only sporadically after 6 weeks, though incidence increased particularly at the high dose during the final 6 months of the study. Other clinical effects 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 clearly 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/ $\mu$ l) and 105 (845, 957, 1185, 1568\* th/ $\mu$ l), 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\*  $\mu$ mol/ml). Statistically significant reductions in brain cholinesterase activities were noted for mid and high dose males and females at week 53 ( $\sigma$ : 86.0, 81.3, 70.7\*, 36.9\*;  $\rho$ : 84.1, 81.1, 73.5\*, 44.6\*  $\mu$ mol/g) and for mid and high dose males and high dose females at week 105 ( $\sigma$ : 59.9, 59.4, 52.0\*, 35.9\*  $\mu$ mol/g;  $\rho$ : 62.2, 58.7, 55.2, 41.0\*  $\mu$ mol/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 ( $\sigma$ : 0/37, 0/31, 0/37, 3/30), enlarged seminal vesicle ( $\sigma$ : 15/37, 12/31, 12/37, 1/30), uterine mass ( $\sigma$ : 8/34, 4/31, 5/32, 0/32), uterine cyst ( $\sigma$ : 17/34, 14/31, 20/32, 6/32), and internal eye opacity ( $\sigma$ : 1/37, 2/31, 1/37, 4/30;  $\sigma$ : 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-8a.

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-8b). 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<sup>8</sup> 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 oncogenic effects, as noted below in vascular tissue (many organs), kidney and liver.

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

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 vascular neoplasms were present in some animals) was 2/66, 6/66, 10/69\* and 10/68\* at increasing doses (\* $p < 0.05$ ; see footnote d, Table III-8c). 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 also showed 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, clinical signs and evidence from a mouse pharmacokinetic study that metabolism was altered at the high dose (Valles, 1999). 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-8a. Effects of carbaryl on organ weights; 2 year CD-1 mouse study (Hamada, 1993b)

	Carbaryl dose, males (ppm) <sup>a</sup>				Carbaryl dose, females (ppm) <sup>a</sup>			
	0	100	1000	8000	0	100	1000	8000
<b>Body wts. (g)</b>								
<b>Week 53</b>	39.6	40.9	37.1	36.0*	31.7	35.4*	35.6*	32.1
<b>Week 105</b>	37.3	37.6	38.0	32.5*	32.4	34.0	32.6	27.6*
<b>Brain (g) (wk. 105)</b>	0.49	0.50	0.48	0.50	0.51	0.51	0.52	0.48
<b>Lung (wk. 105)</b>								
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*
<b>Liver/gall bladder (wk 105)</b>								
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
<b>Kidney (wk 105)</b>								
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
<b>Ovary (wk 53)</b>								
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

<sup>a</sup> 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-8b. Non-neoplastic changes in CD-1 mice exposed over a 2-yr period to dietary carbaryl (Hamada, 1993b)

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

<sup>a</sup> 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.

<sup>b</sup> T, terminal sacrifice; U, unscheduled deaths; I, interim sacrifice

<sup>c</sup> Totals exclude the interim sacrifices.

Table III-8c. Neoplastic changes in CD-1 mice exposed over a 2-yr period to dietary carbaryl (Hamada, 1993b)

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

\*, \*\*: 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.

<sup>a</sup> 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.

<sup>b</sup> T, terminal sacrifice; U, unscheduled deaths; I, interim sacrifice

<sup>c</sup> Totals exclude the interim sacrifices.

<sup>d</sup> 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”.

<sup>e</sup> Includes one animal with multiple kidney tubule cell adenomas.

<sup>f</sup> Includes one animal with multiple hepatocellular adenomas.

<sup>g</sup> Includes two animals each at the mid and high doses with multiple hepatocellular adenomas.

<sup>h</sup> This animal exhibited multiple hepatocellular carcinomas.

<sup>i</sup> Because of the appearance of hepatocellular adenomas in the male interim sacrifices, all animals were considered to be “at risk”.

<sup>j</sup> 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  $1.20 \pm 1.75$  per 1000 cells (range of 0 to 4), while treated tissue had  $3.90 \pm 2.18$  (range of 1 to 7). For female hepatocytes, the control mean was  $4.60 \pm 7.68$  (range of 0 to 23) and treated  $8.33 \pm 3.84$  (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), showed 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  $1.0 \pm 0.0$  to  $1.2 \pm 2.2$ ; Expt. #2 from  $1.1 \pm 0.0$  to  $1.3 \pm 0.3$ ). 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  $3.7 \pm 0.6$  to  $5.7 \pm 1.4$  ( $p < 0.05$ ). 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  $3.06 \pm 0.14$  pm/mg protein to  $3.86 \pm 0.11$  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  $\mu$ g benzo[a]pyrene (BP), 3x/wk; Group III - 100 mg/kg carbaryl, 3x/wk; Group IV - vehicle control, 100  $\mu$ l 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  $\mu$ g 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  $\mu$ g TPA, 3x/wk; Group IV - single treatment with 52  $\mu$ g DMBA, followed 1 week later by 5  $\mu$ g TPA, 3x/wk; Group V - multiple treatments (3x/wk for 3 weeks) with 100 mg/kg carbaryl, followed 1 week later by 100  $\mu$ l acetone, 3x/wk; Group VI - multiple treatments (3x/wk for 3 weeks) with 100  $\mu$ l acetone, followed 1 week later by 5  $\mu$ g 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  $\mu$ g DMBA, followed 1 week later by 100 mg/kg carbaryl, 3x/wk; Group III - single treatment with 52  $\mu$ g DMBA, followed 1 week later by 5  $\mu$ g TPA, 3x/wk; Group IV - single treatment with 100  $\mu$ l acetone, followed 1 week later by 100 mg/kg carbaryl, 3x/wk; Group V - single treatment with 52  $\mu$ g DMBA, followed 1 week later by 100  $\mu$ l 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/ $\mu$ l) and 52 (10.3, 11.1, 9.9, 15.2\* Th/ $\mu$ l), and in segmented neutrophil counts at week 52 (7.0, 8.2, 7.5, 11.4\* Th/ $\mu$ l). 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-9). 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-9. Suppression of cholinesterase activities in beagle dogs by dietary carbaryl; 1-yr study (Hamada, 1987)

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

\*p<0.05

<sup>a</sup> 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.

<sup>b</sup> Percent of concurrent control activities.

Table III-10. NOEL and LOEL values for subchronic and chronic toxicity studies on carbaryl

| Species, strain                              | Study type & exposure regimen | Effects at LOEL                                                                       | NOEL                                                                             | LOEL                                                                               | Reference <sup>f</sup>                 |
|----------------------------------------------|-------------------------------|---------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|----------------------------------------|
| <b>Subchronic studies:</b>                   |                               |                                                                                       |                                                                                  |                                                                                    |                                        |
| Dog, Beagle                                  | 5-wk dietary                  | none                                                                                  | 125 ppm <sup>a</sup> (M: 3.83 mg/kg/day; F: 4.11 mg/kg/day)                      | >125 ppm <sup>1</sup> (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. <sup>b</sup>                                                      | 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 <i>et al.</i> (1974) |
| Rat, Sprague-Dawley                          | 4-wk dermal <sup>c</sup>      | systemic: no systemic toxicity<br>local: atonia<br>ChE: inhibition of brain & RBC ChE | systemic: 100 mg/kg/day <sup>a</sup><br>local: 50 mg/kg/day<br>ChE: 20 mg/kg/day | systemic: >100 mg/kg/day <sup>a</sup><br>local: 100 mg/kg/day<br>ChE: 50 mg/kg/day | Supplemental Austin (2002a)            |
| <b>Chronic studies:</b>                      |                               |                                                                                       |                                                                                  |                                                                                    |                                        |
| Dog, Beagle                                  | 1-yr dietary <sup>e</sup>     | ↓ 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 <sup>d</sup>           | 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);             |

<sup>a</sup> Highest dose tested.

<sup>b</sup> This LOEL determinant was considered conditional - there was no histopathology done to determine if the increased relative liver weight was adverse in nature.

<sup>c</sup> Dermal exposure was for 5 days/wk, 6 hr/day.

<sup>d</sup> There was also an increase in hemangiosarcomas at all doses in the Hamada (1993b) mouse study.

<sup>e</sup> The 1-yr dog dietary study represents the critical chronic study. Benchmark dose calculations indicate an LED<sub>10</sub> of 0.5 mg/kg/day.

<sup>f</sup> 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

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 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  $\alpha$ -naphthol (1-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 appear in Table III-11. Study summaries can be found in DPR's dietary risk characterization document on carbaryl (DPR, 2010: <http://www.cdpr.ca.gov/docs/risk/rcd/carbaryl.pdf>).

Table III-11. Genotoxic effects of carbaryl

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

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

<sup>a</sup> 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.

<sup>b</sup> 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 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 sperm toxicity parameters, including 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 naphthalene. 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.

These studies are summarized below and in Table III-13.

### 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  $6.3 \pm 3.9$  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<sup>3</sup> (mean = 4.9 mg/m<sup>3</sup>), 22 samples from the distribution area ranged between 0.03 and 1.8 mg/m<sup>3</sup> (mean = 0.347 mg/m<sup>3</sup>), and 36 personal monitoring samples from the same area ranged between 0.0 and 1.8 mg/m<sup>3</sup> (mean = 0.439 mg/m<sup>3</sup>).

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<sup>6</sup>/ml (n=34), compared to 140.7x10<sup>6</sup>/ml (n=48) for the exposed group. Age-matched groups (18-40 yr) showed counts of 124.7x10<sup>6</sup>/ml (controls, n=33) vs. 120.3x10<sup>6</sup>/ml (exposed, n=26). However, a non-statistically significant elevation of oligospermic individuals (*i.e.*, those with sperm count < 20x10<sup>6</sup>/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 ( $41.2\pm 2.5\%$  in controls vs.  $52.6\pm 3.6\%$  in high exposure group;  $p<0.01$ ), there was no statistically significant difference in percent sperm with two fluorescent bodies ( $0.8\pm 0.2\%$  in controls vs.  $1.0\pm 0.3\%$  in exposed) nor in percent sperm with one fluorescent body ( $44.7\pm 0.9\%$  in controls vs.  $44.3\pm 1.0\%$  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  $80\times 10^6$  sperm/ml and percent abnormal sperm. There were 18 men in the sub- $80\times 10^6$ /ml category, showing  $64.0\pm 3.8\%$  abnormal sperm vs. 29 men in the plus- $80\times 10^6$  sperm/ml category, who showed  $43.6\pm 1.8\%$  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.

\*\*\*\*\*

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 were 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 naphthalene) 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" ( $STR = [VSL \div VAP] \times 100$ ) and "linearity" ( $LIN = [VSL \div VCL] \times 100$ ).

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<sup>#</sup>, 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<sup>#</sup>, 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  $36.1 \pm 5.6$  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  $\mu$ l 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  $\mu$ m 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  $20.50 \pm 6.71\%$  among internal controls,  $15.92 \pm 7.58\%$  among external controls and  $25.25 \pm 4.90\%^{**}$  among the carbaryl-exposed group ( $^{**}p < 0.01$  compared to external controls). This was due primarily to statistically significant increases in head abnormalities ( $9.85 \pm 5.12\%$  vs.  $7.56 \pm 3.61\%$  vs.  $11.72 \pm 4.93\%^{*}$ ;  $p < 0.05$ ) and tail abnormalities ( $8.73 \pm 4.10\%$  vs.  $6.60 \pm 4.78\%$  vs.  $10.82 \pm 3.09\%^{**}$ ).

TUNEL assays showed a statistically significant increase in the percentage of cells with fragmented DNA in the carbaryl-exposed population:  $13.36 \pm 12.17\%$  in internal controls,  $13.92 \pm 7.15\%$  in external controls,  $21.04 \pm 8.88\%^{*}$  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  $0.280 \pm 0.076\%$ ,  $0.177 \pm 0.080\%$  and  $0.281 \pm 0.102\%^{**}$  ( $p < 0.01$  compared to external controls). The percentage of YY18 sperm was  $0.134 \pm 0.052\%$ ,  $0.079 \pm 0.042\%$  and  $0.185 \pm 0.083\%^{**}$  ( $p < 0.01$  compared to external controls;  $p < 0.05$  compared to internal controls). The percentage of X1818 sperm was  $0.093 \pm 0.053\%$ ,  $0.051 \pm 0.028\%$  and  $0.113 \pm 0.070\%^{*}$  ( $p < 0.05$  compared to external controls). The percentage of Y1818 was  $0.076 \pm 0.031\%$ ,  $0.052 \pm 0.043\%$  and  $0.119 \pm 0.055\%^{**}$  ( $p < 0.01$  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  $0.383 \pm 0.099\%$ ,  $0.277 \pm 0.077\%$  and  $0.426 \pm 0.174\%^{**}$  ( $p < 0.01$  compared to external controls). The percentage of sperm nullisomic for chromosome 18 was  $0.222 \pm 0.062\%$ ,  $0.139 \pm 0.043\%$  and  $0.268 \pm 0.126\%^{*}$  ( $p < 0.05$  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<sub>0</sub> 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<sub>1</sub> 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<sub>0</sub> or F<sub>1</sub> parental epididymal sperm counts, motility, morphology, homogenization-resistant spermatid head counts, daily sperm production or efficiency of daily sperm production.

Food consumption among F<sub>0</sub> 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<sub>0</sub> females showed no differences in food consumption. Similar to the F<sub>0</sub> males, F<sub>1</sub> males showed lower g/day food consumption at 1500 ppm, though g/kg/day consumption was actually higher. The same was true for F<sub>1</sub> females.

Mean pre-breeding body weights were consistently statistically suppressed at 1500 ppm among F<sub>0</sub> and F<sub>1</sub> animals of both sexes. These were largely reflective of statistical decrements in weight gain during the first pre-breeding week of exposure (Table III-12). There was also some indication that weight gains among the 300 ppm animals were suppressed, particularly among F<sub>1</sub> males. Maternal body weight gains were suppressed at 1500 ppm in both the F<sub>0</sub> and F<sub>1</sub> generations, though lactational weight gains appeared little affected, or were increased, by carbaryl exposure. Mean F<sub>1</sub> and F<sub>2</sub> pup body weights tended to be suppressed at 1500 ppm, particularly as the lactational period progressed (Table III-12, 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<sub>1</sub>-day 14 pups, F<sub>2</sub>-day 4 pups and F<sub>2</sub>-day 7 pups (Table III-12). The mean number of live pups / litter, F<sub>1</sub>-pnd 4 (pre-cull) did not appear affected. However, the same parameter for the F<sub>2</sub> pups was statistically depressed at the mid and high doses (Table III-12).

Both male and female F<sub>1</sub> 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-12). Because the F<sub>2</sub> pups were sacrificed at weaning, similar measurements were not made for them. However, anogenital distance measurements on F<sub>2</sub> pups did not suggest an effect.

Necropsies on parental animals and pups, F<sub>0</sub>-F<sub>1</sub>-F<sub>2</sub>, 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<sub>2</sub> 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-12 . Effect of dietary carbaryl on reproductive parameters in CD rats (Tyl *et al.*, 2001)

|                                                            | Carbaryl concentration in diet (ppm) <sup>a</sup> |       |       |          |
|------------------------------------------------------------|---------------------------------------------------|-------|-------|----------|
|                                                            | 0                                                 | 75    | 300   | 1500     |
| <b>Body wt. gain, pre-breeding days 0-7 (g)</b>            |                                                   |       |       |          |
| F <sub>0</sub> ♂                                           | 58.9                                              | 58.6  | 54.6  | 45.2**   |
| F <sub>0</sub> ♀                                           | 24.5                                              | 24.5  | 23.4  | 17.9***  |
| F <sub>1</sub> ♂                                           | 64.6                                              | 63.1  | 58.6* | 54.2**   |
| F <sub>1</sub> ♀                                           | 40.1                                              | 40.4  | 37.8  | 36.3*    |
| <b>Body wt. gain, gestation days 0-20 (g)</b>              |                                                   |       |       |          |
| F <sub>0</sub> ♀                                           | 138.1                                             | 130.2 | 132.0 | 121.0*** |
| F <sub>1</sub> ♀                                           | 140.1                                             | 137.2 | 134.3 | 115.3*** |
| <b>Body wt. gain, postnatal days 0-21 (g)</b>              |                                                   |       |       |          |
| F <sub>0</sub> ♀                                           | 9.3                                               | 3.3   | 12.6  | 14.4     |
| F <sub>1</sub> ♀                                           | 8.5                                               | 5.7   | 10.8  | 15.8     |
| <b>Pup body wts. (g)</b>                                   |                                                   |       |       |          |
| F <sub>1</sub> , postnatal day 0                           | 6.34                                              | 6.68  | 6.41  | 6.09     |
| F <sub>1</sub> , postnatal day 21                          | 48.79                                             | 49.46 | 50.73 | 43.46*** |
| F <sub>2</sub> , postnatal day 0                           | 6.27                                              | 6.51  | 6.58  | 6.00     |
| F <sub>2</sub> , postnatal day 21                          | 50.91                                             | 52.30 | 49.68 | 40.39*** |
| <b>Pup survival index (%)</b>                              |                                                   |       |       |          |
| F <sub>1</sub> , 4-day                                     | 98.4                                              | 99.1  | 95.0  | 98.1     |
| F <sub>1</sub> , 7-day                                     | 99.7                                              | 100.0 | 99.6  | 99.3     |
| F <sub>1</sub> , 14-day                                    | 99.7 <sup>+</sup>                                 | 100.0 | 99.2  | 95.4     |
| F <sub>2</sub> , 4-day                                     | 98.3 <sup>++</sup>                                | 98.7  | 92.0  | 88.9     |
| F <sub>2</sub> , 7-day                                     | 100.0 <sup>+</sup>                                | 99.6  | 96.0  | 93.0     |
| F <sub>2</sub> , 14-day                                    | 98.6                                              | 99.6  | 94.8  | 96.6     |
| <b>Mean live pups / litter (pre-cull)</b>                  |                                                   |       |       |          |
| F <sub>1</sub> , postnatal day 4                           | 13.9                                              | 13.3  | 13.9  | 14.3     |
| F <sub>2</sub> , postnatal day 4                           | 15.4 <sup>++</sup>                                | 13.9  | 12.7* | 12.5**   |
| <b>Pup developmental indicators</b>                        |                                                   |       |       |          |
| F <sub>1</sub> ♂, day of preputial separation <sup>b</sup> | 41.6                                              | 41.5  | 41.7  | 43.7**   |
| F <sub>1</sub> ♀, day of vaginal opening <sup>b</sup>      | 30.6                                              | 31.3  | 31.3  | 32.0**   |

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

<sup>+</sup> , <sup>++</sup>: p<0.05, 0.01 (trend test)

<sup>a</sup> Calculated carbaryl intakes for the F<sub>0</sub> and F<sub>1</sub> 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.

<sup>b</sup> Because the F<sub>2</sub> pups were sacrificed at weaning, similar developmental measurements were not made for them.

#### 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<sub>50</sub> 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 <sup>131</sup>I in the thyroid was also impaired by carbaryl exposure, indicated "by a reduction in the rate of absorption and excretion of <sup>131</sup>I and its rather low recovery, in comparison with the controls. Thus at the two lowest dosage levels, <sup>131</sup>I-absorption reached a peak within the first 4-6 hr and represented on average 16% of the administered <sup>131</sup>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 <sup>131</sup>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 <sup>131</sup>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 <sup>131</sup>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,  $2.96 \pm 0.17$  vs.  $3.49 \pm 0.13^*$  nm/min/mg protein), adenosine triphosphatase ( $72.31 \pm 1.61$  vs.  $83.43 \pm 3.83^*$  nm/min/mg protein); liver - glucose-6-phosphatase ( $83.08 \pm 4.25$  vs.  $100.16 \pm 5.41^*$  nm/min/mg protein); blood - AChE ( $8.15 \pm 0.45$  vs.  $5.30 \pm 0.56^{***}$   $\mu\text{mol/ml/10 min}$ ); brain - AChE ( $0.96 \pm 0.02$  vs.  $0.85 \pm 0.04^*$   $\mu\text{mol/100 } \mu\text{l of 10\% homogenate/10 min}$ ).

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  $\text{LD}_{50}$ ), 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.



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<sup>\*\*\*</sup> and ~-9<sup>\*\*\*</sup> g, respectively; <sup>\*\*\*</sup>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<sup>\*\*</sup> and ~-1.5 g<sup>\*\*\*</sup>; <sup>\*\*</sup>, <sup>\*\*\*</sup> 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 induced parallel maternal toxicity.



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 epididymal 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  $\gamma$ -glutamyl transpeptidase (marker enzyme for Sertoli cell function) were statistically increased at both doses (LDH: 244, 390\*, 500\* nmol/min/mg;  $\gamma$ GT: 23.2, 37.3\*, 58.3\*).

Total epididymal sperm counts and percent sperm motilities were statistically decreased at both doses (sperm counts/epididymis:  $10 \times 10^7$ ,  $6 \times 10^7$ \*,  $4 \times 10^7$ \*; 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 Drucker 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,  $8.0 \times 10^7$ ,  $8.2 \times 10^7$ ,  $6.0 \times 10^7$ \* and  $5.0 \times 10^7$ \* ( $p < 0.05$ ). In older rats they were  $8.0 \times 10^7$ ,  $8.5 \times 10^7$ ,  $7.0 \times 10^7$ \* and  $6.0 \times 10^7$ \*. 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<sub>3a</sub> and F<sub>3b</sub> 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<sub>2</sub> generation. Statistically significant effects at other doses were less clearly related to exposure<sup>9</sup>. 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

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<sup>9</sup> Data for the parameters discussed in the summary of the Collins *et al.* study in gerbils are as follows:

Fertility index at increasing doses, 1<sup>st</sup> mating-2<sup>nd</sup> mating, F<sub>1</sub> generation: 95%-89%, 83%-84%, 77%\*-78%, 93%-79%, 83%-64%; F<sub>2</sub> generation: 98%-100%, 97%-93%, 93%-93%, 90%-93%, 60%\*-33%\*\*; F<sub>3</sub> generation: 100%-98%, 87%\*-81%\*, 93%-89%, 95%-81%\*, 50%\*-0%\*\*; \*p<0.05, \*\*p<0.01.

Mean litter size, 1<sup>st</sup> mating-2<sup>nd</sup> mating, F<sub>1</sub> generation: 4.90-4.26, 4.27-3.60, 3.97-4.13, 4.50-3.61, 4.00-3.00; F<sub>2</sub> generation: 5.70-5.23, 5.07-4.55, 4.47\*-4.79, 4.50\*-5.00, 3.20\*-1.33\*; F<sub>3</sub> 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, 1<sup>st</sup> mating-2<sup>nd</sup> mating, F<sub>1</sub> generation: 4.67-4.13, 4.13-3.56, 3.67-4.00, 3.80-3.36, 3.06\*-2.57; F<sub>2</sub> generation: 5.60-5.10, 4.80-4.24, 3.90\*\*-4.50, 4.03\*\*-4.56, 3.00\*\*-1.33\*\*; F<sub>3</sub> 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, 1<sup>st</sup> mating-2<sup>nd</sup> mating, F<sub>1</sub> generation: 3.55-3.32, 2.37-2.68, 2.20\*-2.57, 2.23\*-1.93\*, 0.94\*\*-1.00\*\*; F<sub>2</sub> generation: 4.70-4.36, 3.57\*-3.31\*, 2.30\*\*-2.46\*\*, 1.97\*\*-2.30\*\*, 1.40\*\*-0.67\*\*; F<sub>3</sub> 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, 1<sup>st</sup> mating-2<sup>nd</sup> mating, F<sub>1</sub> generation: 3.30-3.16, 2.33-2.48, 2.07-2.43, 2.10-1.86, 0.56\*\*-0.79\*\*; F<sub>2</sub> generation: 4.17-4.26, 3.43-3.17, 2.17\*\*-2.18\*\*, 1.60\*\*-2.15\*\*, 1.20\*\*-0.67\*\*; F<sub>3</sub> 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, 1<sup>st</sup> mating-2<sup>nd</sup> mating, F<sub>1</sub> generation: 15.1-14.5, 15.4-15.6, 11.1\*-14.0, 13.1\*-13.6, 13.2\*\*-13.4; F<sub>2</sub> 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<sub>3</sub> 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.

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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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  $\mu^3$  in controls, 408.61  $\mu^3$  in treated animals; nucleus paraventricularis: 312.18  $\mu^3$  in controls, 359.34  $\mu^3$  in treated animals) and in the number of Gomori stained-positive glial cells per 0.076 mm<sup>2</sup> 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 BenomyI) 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<sub>50</sub> for this compound (LD<sub>50</sub> = 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-13. 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
<b>CD rat</b>	2-gen. repro., dietary	parental: ↓ wt. gains repro.: no effect at HDT <sup>a</sup> pup: ↑ F <sub>2</sub> mortality	parental: 5-6 mg/kg/day repro.: >92-136 mg/kg/day pup: 75 ppm <sup>b</sup>	parental: 21-36 mg/kg/day repro.: >92-136 mg/kg/day pup: 300 ppm <sup>b</sup>	<i>Acceptable</i> Tyl <i>et al.</i> (2001)
<b>Rat (strain not identified)</b>	12-month oral gavage	↓ sperm motility, effects on hypophyseal & thyroid function, and adrenal & thyroid histopath.	<7 mg/kg/day (LDT) <sup>a</sup>	7 mg/kg/day (LDT) <sup>a</sup>	<i>Supplemental</i> Shtenberg & Rybakova (1968)
<b>Wistar rat (♂ only)</b>	90-day oral gavage	changes in testicular enzyme levels, and sperm / testicular histopath.	<50 mg/kg/day (LDT) <sup>1</sup>	50 mg/kg/day (LDT) <sup>1</sup>	<i>Supplemental</i> Pant <i>et al.</i> (1995)
<b>Druckrey rat (♂ only)</b>	60-day oral gavage	damage to the ♂ reproductive system	25 mg/kg/day	50 mg/kg/day	<i>Supplemental</i> Pant <i>et al.</i> (1996)
<b>Mongolian gerbil</b>	100-day dietary	repro.: ↓ # liveborn pups, ↓ # survivors (days 4-21), ↓ weanling weights	repro.: <2000 ppm (~160 mg/kg/day) (LDT) <sup>a, c</sup>	repro.: 2000 ppm (~160 mg/kg/day) (LDT) <sup>1,3</sup>	<i>Supplemental</i> Collins <i>et al.</i> (1971)

<sup>a</sup> HDT, high dose tested; LDT, low dose tested

<sup>b</sup> This value is expressed as a dietary concentration because it was not possible to determine actual carbaryl intake in the pups.

<sup>c</sup> 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 related to 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-15.

### **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 7<sup>th</sup> cervical centrum, incomplete ossification of the 5<sup>th</sup> sternebra and non ossification of the 1<sup>st</sup> 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 was 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 ( $\sigma$ : 52.69, 51.29, 50.48, 47.44\* g;  $\text{♀}$ : 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 sternbrae 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.



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:  $-0.03 \pm 0.09$  kg vs.  $-0.15 \pm 0.10^*$  kg; controls vs. 200 mg/kg/day,  $-0.03 \pm 0.07$  kg vs.  $-0.31 \pm 0.10^*$  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,  $0.8 \pm 1.2$  vs.  $1.3 \pm 2.8$ ; control vs. 200 mg/kg/day,  $0.5 \pm 1.1$  vs.  $1.5 \pm 1.9$ ).

Fetal body weights were reduced at both doses, though only the lower dose achieved statistical significance (control vs. 150 mg/kg/day,  $37.9 \pm 5.4$  g vs.  $34.0 \pm 3.4^*$  g; control vs. 200 mg/kg/day,  $39.2 \pm 4.2$  g vs.  $36.7 \pm 3.8$  g). Fetuses also were slightly smaller in size, though not by statistically significant margins (fetal crown-rump length, control vs. 150 mg/kg/day,  $93.5 \pm 5.9$  mm vs.  $91.1 \pm 3.6$  mm; control vs. 200 mg/kg/day,  $96.3 \pm 4.1$  mm vs.  $93.8 \pm 3.6$  mm).

Omphalocele<sup>10</sup> 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:  $2 \pm 1$ ,  $2 \pm 2$  and  $0 \pm 2^*$  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:  $2 \pm 1$  and  $1 \pm 2$  g), but did between gd 10-15 ( $11 \pm 4$  and  $7 \pm 4^*$  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

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<sup>10</sup> 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 protruding bowel being covered only by a thin transparent membrane composed of amnion and peritoneum" (Dorland's Illustrated Medical Dictionary, 26<sup>th</sup> edition, page 921). It is considered to be an external malformation.

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 carbaryl ( $1.02 \pm 0.12$  vs.  $0.80 \pm 0.14^*$  g), as was the fetal crown-rump length ( $24.1 \pm 1.3$  mm vs.  $22.2 \pm 1.8^*$  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-14 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  $\Rightarrow$  8 pups; mid dose female #5575  $\Rightarrow$  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, intussusception 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 13<sup>th</sup> 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-14. 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                                       |
| <b>Maternal</b>                                                     |                           |                               |                                           |                                            |
| Mated females                                                       | 12                        | 12                            | 12                                        | 12                                         |
| Number pregnant                                                     | 9                         | 7                             | 9                                         | 9                                          |
| Maternal deaths                                                     | 0                         | 1 <sup>a</sup>                | 1 <sup>b</sup>                            | 1 <sup>b</sup>                             |
| <b>Offspring</b>                                                    |                           |                               |                                           |                                            |
| 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<br>[# available litters]                   | 1/45 (2%)<br>[1/9] (11%)  | 3/33 (9%)<br>[3/7] (43%)      | 16/46** (35%)<br>[6/9]* (67%)             | 14/57** (25%)<br>[5/9] (56%)               |
| Deaths (0-24 hr) / total pups<br>[# available litters]              | 1/44 (2%)<br>[1/9] (11%)  | 0/30 (0%)<br>[0/7] (0%)       | 0/30 (0%)<br>[0/7 <sup>c</sup> ] (0%)     | 1/43 (2%)<br>[1/8 <sup>d</sup> ] (13%)     |
| Deaths (24-48 hr) / total pups<br>[# available litters]             | 0/43 (0%)<br>[0/9] (0%)   | 2/30 (7%)<br>[1/7] (14%)      | 2/30 (7%)<br>[2/7 <sup>3</sup> ] (29%)    | 4/42 (10%)<br>[3/8 <sup>d</sup> ] (38%)    |
| Deaths (48 hr - 6 wk weaning) / total pups<br>[# available litters] | 5/43 (12%)<br>[4/9] (44%) | 10/28* (36%)<br>[3/7] (43%)   | 9/28* (32%)<br>[5/7 <sup>3</sup> ] (71%)  | 12/38* (32%)<br>[5/8 <sup>d</sup> ] (63%)  |
| Deaths (24 hr - 6 wk weaning) / total pups<br>[# available litters] | 5/43 (12%)<br>[4/9] (44%) | 12/30** (40%)<br>[4/7] (57%)  | 11/30* (37%)<br>[5/7 <sup>3</sup> ] (71%) | 16/42** (38%)<br>[6/8 <sup>d</sup> ] (75%) |
| <i>Removing affected litters:</i> <sup>e</sup>                      |                           |                               |                                           |                                            |
| Deaths (24 hr - 6 wk weaning) / total pups<br>[# available litters] | 5/39 (13%)<br>[4/8] (50%) | 10/25* (40%)<br>[3/6] (50%)   | 7/15* (47%)<br>[3/3] (100%)               | 4/18 (22%)<br>[2/4] (50%)                  |
| Total pup mortality<br>[# available litters]                        | 7/45 (16%)<br>[6/9] (67%) | 15/33** (45%)<br>[7/7] (100%) | 27/46** (59%)<br>[9/9] (100%)             | 31/57** (54%)<br>[9/9] (100%)              |

<sup>a</sup> This low-dose mother was killed *in extremis* on day 48 with convulsions and in poor general health.

<sup>b</sup> The mid and high-dose maternal deaths occurred at parturition on days 54 and 61, respectively.

<sup>c</sup> 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.

<sup>d</sup> 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.

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

<sup>a</sup> HDT, high dose tested; LDT, low dose tested

<sup>b</sup> Dietary exposure probably continued through weaning, 8 weeks *post partum*, though the report was not explicit on this point.

<sup>c</sup> 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<sub>1</sub> generation, consisting of 3 males and 3 females, was weaned at day 21.

F<sub>0</sub> 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<sub>1</sub> adult generation.

F<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> adults from the control and high doses.

**Results, F<sub>0</sub> 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-16a). 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. 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-16b). 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<sub>1</sub> pups.** There were no unambiguous effects of carbaryl in the F<sub>1</sub> 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<sub>1</sub> pups sacrificed on ppd 11 and F<sub>1</sub> 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<sub>1</sub> adults.** There were no clear effects of carbaryl in the F<sub>1</sub> 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<sub>10</sub> of 0.25 mg/kg (see section IV.1.a.). Consequently, both 1 mg/kg and 0.25 mg/kg might be used to gauge the potential acute risk from exposure to carbaryl (see Hazard Identification and Risk Appraisal sections below).

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-16a. Selected functional observational battery observations in F<sub>0</sub> females during the period of dosing with carbaryl (gd 6 - ppd 10), F<sub>0</sub> females (Robinson and Broxup, 1997)

|                                     | Carbaryl dose (mg/kg/day) |              |               |                |
|-------------------------------------|---------------------------|--------------|---------------|----------------|
|                                     | Control                   | 0.1          | 1.0           | 10.0           |
| <b><u>Slight hypotonic gait</u></b> |                           |              |               |                |
| gd 6 <sup>a,c</sup>                 | 6/23 (26.1) <sup>b</sup>  | 7/26 (26.9)  | 7/26 (26.9)   | 11/23 (47.8)   |
| gd 9 <sup>c</sup>                   | 7/26 (26.9)               | 2/26 (7.7)   | 10/26 (38.5)  | 11/26 (42.3)   |
| gd 12 <sup>c</sup>                  | 5/26 (19.2)               | 5/26 (19.2)  | 13/26 (50.0)* | 11/26 (42.3)   |
| gd 15 <sup>c</sup>                  | 7/25 (28.0)               | 11/26 (42.3) | 14/26 (53.8)  | 10/26 (38.5)   |
| gd 18 <sup>c</sup>                  | 5/25 (20.0)               | 10/26 (38.5) | 11/26 (42.3)  | 15/26 (57.7)** |
| gd 20 <sup>c</sup>                  | 9/25 (36.0)               | 5/26 (19.2)  | 11/26 (42.3)  | 13/26 (50.0)   |
| ppd 4 <sup>a</sup>                  | 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)    |
| <b><u>Slight ataxic gait</u></b>    |                           |              |               |                |
| 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)       |
| <b><u>Slight tremors</u></b>        |                           |              |               |                |
| 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)       |

|                                                     |             |              |               |                 |
|-----------------------------------------------------|-------------|--------------|---------------|-----------------|
| <b><u>Pinpoint pupils</u></b>                       |             |              |               |                 |
| 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)        |
| <b><u>Moderate dilation of pupils</u></b>           |             |              |               |                 |
| 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) ✕      |
| gd 12                                               | 2/26 (8)    | 0/26 (0) ✕   | 2/26 (8)      | 0/26 (0) ✕✕     |
| gd 15                                               | 2/25 (8)    | 4/26 (15)    | 2/26 (8)      | 0/26 (0) ✕✕     |
| 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) ✕✕     |
| 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)        |
| <b><u>Signs (per animal basis) <sup>d</sup></u></b> |             |              |               |                 |
| 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.

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

<sup>a</sup> Abbreviations: gd, gestation day; ppd, *post partum* day

<sup>b</sup> Numbers in parentheses are the incidence rates expressed in percentages.

<sup>c</sup> 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.

<sup>d</sup> 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-16b. RBC, plasma and brain cholinesterase activities in F<sub>0</sub> 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                 |
| <b><u>RBC ChE (U/L)</u></b>    |                           |                       |                       |                      |
| gd 6 <sup>a</sup>              | 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 <sup>a</sup>             | 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) |
| <b><u>Plasma ChE (U/L)</u></b> |                           |                       |                       |                      |
| gd 6 <sup>a</sup>              | 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 <sup>a</sup>             | 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)   |
| <b><u>Brain ChE (U/g)</u></b>  |                           |                       |                       |                      |
| ppd 10                         | 5.9±0.04                  | 6.2±0.22 (104.7)      | 5.8±0.16 (97.9)       | 3.4±0.58** (58.2)    |

<sup>a</sup> Abbreviations: gd, gestation day; ppd, *post partum* day  
 Parenthetical values represent percent of concurrent controls.

## 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 Ingredient 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.

Health risks associated with exposure to carbaryl-associated 1-naphthol were not factored into the MOE or cancer risk calculations. 1-naphthol exhibited a substantially higher acute LD<sub>50</sub> 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 carbaryl's acute, subchronic and chronic carbaryl critical endpoints. Finally, carbaryl-associated 1-naphthol air levels were not known. It is recognized that exclusion of this degradate from the risk analysis may result in underestimation of health risks associated with carbaryl usage.

**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 <sup>14</sup>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 centrifugation 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<sub>50</sub>, 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 histopathologic 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 histopathologic 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 glandular 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 clinical 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<sup>9</sup> / 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<sup>9</sup> / 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<sup>2</sup> 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<sup>2</sup>. 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 LD<sub>50</sub>, 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 LD<sub>50</sub>, 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 absence 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)

**Regulatory limits.** The OSHA PEL (permissible exposure limit) for 1-naphthol (inert or nuisance dust, respirable fraction, TWA) is 5 mg/m<sup>3</sup> and for 1-naphthol (inert or nuisance dust, total dust, TWA) is 15 mg/m<sup>3</sup>. The ACGIH TLV (TWA) is 10 mg/m<sup>3</sup>

## **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<sub>50</sub> 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).

Like 1-naphthol, methylamine was not factored into the health risk calculations for carbaryl. This was mainly because methylamine levels associated with carbaryl usage were not known.

**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

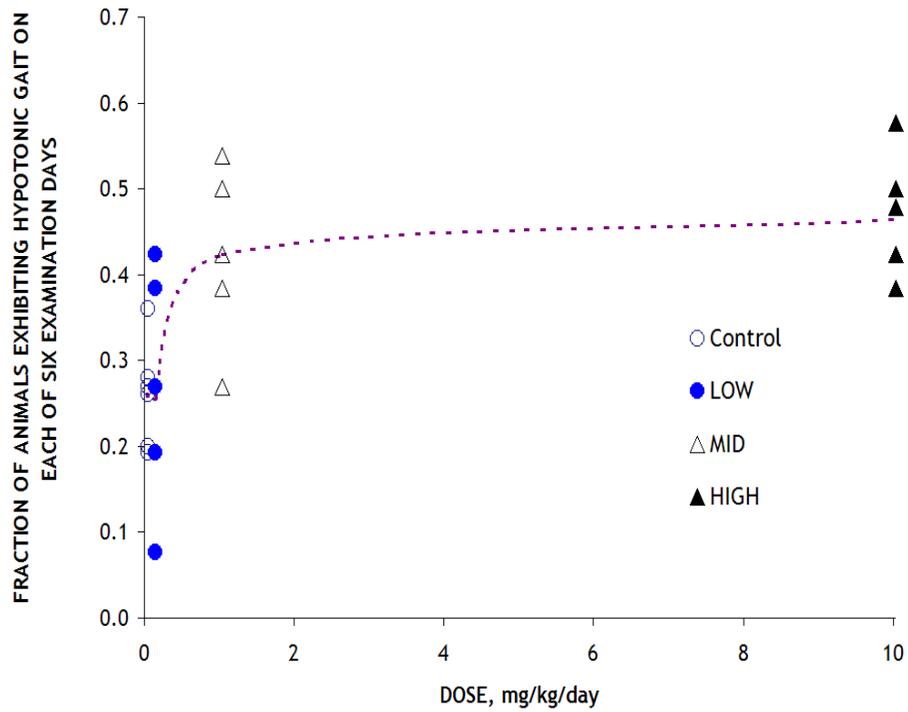
In terms of dose, the most sensitive acute (or short-term) toxicologic endpoints for carbaryl were identified in the gavage developmental neurotoxicity study of Robinson and Broxup (1997). Two plausible acute regulatory endpoint values, including a NOEL and an LED<sub>10</sub>, can be derived from this study.

(1) A NOEL of **1 mg/kg** was based 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. 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. The brain enzyme assays were not done in a timed manner; consequently, the possibility that enzyme inhibition might occur at 1 mg/kg, though earlier in the study than ppd 10, could not be ruled out. Even so, 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<sub>10</sub>) 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 are 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 were a plausible reflection of cholinergic effects even at the mid dose of 1 mg/kg. Figure 2 shows a scatter plot of the data for slight hypotonic gait.

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,

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



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<sup>11</sup> Superimposition of data points resulted in less than six identifiable points / dose in this figure.

but equivalent, acute scenarios<sup>12</sup>. Consequently, the gestational data sets were combined to generate a normalized mean incidence rate, as noted in Table IV-1. 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<sub>10</sub> values higher than a putative effect level at 1 mg/kg. Deletion of the top dose resulted in an appropriate curve fit using the probit algorithm (Appendix I). This was considered an appropriate step since the resultant curve was likely to better represent the response in the dose range relevant to this determination. As noted, the resultant LED<sub>10</sub> was 0.25 mg/kg (ED<sub>10</sub> = 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<sub>10</sub>.

(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<sub>10</sub> (ED<sub>10</sub>) 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

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<sup>12</sup> Post gestational exposures were not included in the analysis because the animals appeared less sensitive following the end of pregnancy.

of 3 mg/kg. This resulted in a LED<sub>10</sub> (ED<sub>10</sub>) 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<sub>10</sub> of 0.25 mg/kg.

Support for the critical acute NOEL and LED<sub>10</sub> 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.

The critical chronic oral LED<sub>10</sub> of 0.5 mg/kg, which was based on brain cholinesterase inhibition in dogs exposed by the dietary route (Hamada, 1997) also supported the critical acute NOEL and LED<sub>10</sub> designations. While Hamada exposed the dogs for one year, the possibility that each exposure was actually a separate acute exposure arose on the basis that carbaryl has a relatively fast rate of detachment from the enzyme. Consequently, 0.5 mg/kg could be viewed as an acute value.

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.

Finally, support for these values was forthcoming from the acute inhalation toxicity study of Weinberg (2008), which established an LED<sub>10</sub> of 9.81 mg/m<sup>3</sup> by BMD analysis. This was based on inhibition of brain cholinesterase activity at a low dose of 10 mg/m<sup>3</sup> (1.2 mg/kg, calculated using the rat default breathing rate of 0.96 m<sup>3</sup>/kg/day) in females after a single 3-hr exposure. The LED<sub>10</sub> (ED<sub>10</sub>) translated to an internal dose of 1.18 (1.70) mg/kg (see calculation in footnote 13 below), essentially equivalent to the critical acute NOEL of 1 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           |
| <b>Slight hypotonic gait</b>        |                           |               |                |                |
| gd 6 <sup>a</sup>                   | 6/23 (26.1) <sup>b</sup>  | 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)   |
| <b>Slight hypotonic gait (mean)</b> | 6.8/26 (26.2)             | 6.7/26 (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.

<sup>a</sup> Abbreviation: gd, gestation day.

<sup>b</sup> Numbers in parentheses are the incidence rates expressed in percentages.

While both critical acute values--the NOEL of 1 mg/kg and the LED<sub>10</sub> of 0.25 mg/kg--are plausible, there is stronger experimental support for the former value. To avoid confusion, this risk assessment uses the NOEL of 1 mg/kg for the generation of acute MOEs in the Risk Characterization section. However, acute MOEs resulting from the use of the 0.25 mg/kg LED<sub>10</sub> are presented and discussed in the Risk Appraisal section.

**b. Subchronic oral toxicity**

The risk from subchronic oral exposure to carbaryl was evaluated using the critical chronic oral value of **0.5 mg/kg/day** (see next section). The only subchronic oral study available, the 5-wk dietary study of Hamada (1991), established a NOEL of at the high dose of 3.83 mg/kg/day, based on a lack of adverse effects at that dose. As 3.83 mg/kg/day was considerably higher than either critical acute oral value presented in part a above (0.25 and 1 mg/kg), it was considered prudent to base the seasonal risk estimation on a value closer to the acute values.

**c. 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-year 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 activities 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<sub>10</sub> 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<sub>10</sub> for brain cholinesterase inhibition in females using the Hill algorithm was **0.5 mg/kg/day** (ED<sub>10</sub> = 1.7 mg/kg/day). This value will be used to evaluate the non-oncogenic risks from annual (*i.e.*, chronic) exposure to carbaryl.

**d. Acute, subchronic and chronic dermal toxicity**

The critical dermal NOEL of **20 mg/kg/day** was based on statistically significant inhibition of brain cholinesterase activity after 4 weeks of daily dermal exposure to 50 mg/kg/day (6-7 hr/day, 5 days/wk) in the 4-wk repeat dose dermal study of Austin (2002a). Brain cholinesterase activities measured on day 26 of that study 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 ( $p < 0.05$ ). This study

was the only one available to assess dermal systemic toxicity, either at the acute or subchronic levels.

**e. Acute inhalation toxicity**

The risk from acute inhalation exposure to carbaryl was evaluated using the critical acute oral NOEL of **1 mg/kg**. While an acute inhalation toxicity study was available (Weinberg, 2008), the non-guideline exposure period of 3 hours and the slightly higher LED<sub>10</sub> of 1.18 (1.70) mg/kg<sup>13</sup> based on brain cholinesterase inhibition in females precluded designation of that study as critical. Nonetheless, the proximity of the calculated internal dose to the critical acute oral NOEL was considered strong support for that value.

**f. Subchronic and chronic inhalation toxicity**

As no inhalation studies were available to allow risk estimation from subchronic or chronic inhalation exposure, the critical chronic oral LED<sub>10</sub> of **0.5 mg/kg/day** from the 1-yr dog dietary study of Hamada (1987) was used (see discussion of this study below). This LED<sub>10</sub> was based on brain cholinesterase inhibition at the low study dose of 3.4 mg/kg/day.

**g. 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<sub>10</sub> 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.

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<sup>13</sup> Internal dose = LED<sub>10</sub> (or ED<sub>10</sub>) x (rat breathing rate x 3 hr / 24 hr) = 9.8 (or 14.15) mg/m<sup>3</sup> x (0.96 m<sup>3</sup>/kg/day x 3/24) = 1.18 (1.70) mg/kg

(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-14). Statistical significance was achieved at the mid and high doses when examined on a per-pup basis, though at the mid dose alone 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-14 with its preceding discussion). Abnormalities - including umbilical hernia, cleft palate, fat-like mass in the heart, intussuption 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-occurrence 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.

#### **h. 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 chromosome 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  $\alpha$ -naphthol (1-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-8c) (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-7c) (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 genotoxicity-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, clinical signs and pharmacokinetic changes - see section III.D.2. for a complete discussion), the mid and low doses did not.

*Hemangiosarcoma* is defined as “a malignant tumor formed by proliferation of endothelial and fibroblastic tissue” (Dorland's Medical Dictionary, 26<sup>th</sup> 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 unclear cellular origin of this type of tumor.

Incidences of hemangiomas and hemangiosarcomas 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-8c). 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-8c)). A USEPA-sponsored reanalysis of 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 likelihood 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 (Valles, 1999), it was excluded from the BMD analysis.

The multistage cancer 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 and based on a 10% response level, was  $1.45 \times 10^{-3} \text{ mg/kg/day}^{-1}$  at the 95% upper bound on dose (*i.e.*, the LED) and  $7.2 \times 10^{-4} \text{ mg/kg/day}^{-1}$  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 allometric dose adjustment factor based on body weight raised to the 3/4ths power (*i.e.*,  $BW^{0.75}$ ) (US EPA, 1992). For rate-related processes, this converts to a ratio of animal-to-human bodyweight raised to the 1/4th power (USEPA, undated). In Hamada (1993b), the mean wk. 53 male body weight was  $38.4 \pm 2.2 \text{ g}$ . Accordingly, the dose adjustment factor was:

$$(BW_A / BW_H)^{0.25} = (0.0384 \text{ kg} / 70 \text{ kg})^{0.25} = 0.153$$

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 multistage cancer model. The resultant human oncogenic potency was  $1.01 \times 10^{-2} \text{ mg/kg/day}^{-1}$  at the 95% upper bound (based on the LED) and  $5.03 \times 10^{-3} \text{ mg/kg/day}^{-1}$  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-7c), 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<sup>14</sup> 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 at the mid and high doses in the week 53 interim sacrifice animals<sup>15</sup>. 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 adequate 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-7a) 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

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<sup>14</sup> 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, 26<sup>th</sup> Edition, 1985; W.B. Saunders Company; p. 31).

<sup>15</sup> 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.

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.

## **B. EXPOSURE ASSESSMENT**

### **1. Introduction**

Estimates of exposure to carbaryl resulting from various occupational, bystander and ambient scenarios were developed by the Worker Health and Safety Branch (WH&S) of DPR. These, along with all of the calculations and assumptions that underlay those calculations, are contained in a companion document to this report entitled Human Exposure Assessment Document for Carbaryl (DPR, 2012). Exposure estimates from that document are summarized below and in the ensuing tables. In addition, this document estimates the potential for dietary exposure. That assessment is found in section 8 below.

### **2. Occupational handler and occupational reentry exposure**

The occupational exposure assessment for carbaryl was divided into the following categories: handlers exposed in agricultural settings, handlers exposed in non-agricultural settings, reentry workers, residential handlers and residential reentry workers. Occupational handler exposure estimates - including short-term absorbed daily dosage (STADD), seasonal average daily dosage (SADD), annual average daily dosage (AADD) and lifetime average daily dosage (LADD) - are summarized in Tables IV-2a and IV-2b. They were calculated by WH&S using assumptions regarding application rates, acres treated/day, dermal and inhalation absorption and default body weight, that are detailed in the exposure assessment document (DPR, 2012). When necessary, surrogate exposure estimates from the Pesticide Handlers Exposure Database (PHED) were used.

Occupational reentry exposures were calculated by WH&S using dislodgeable foliar residues and transfer coefficients, as noted in that document.

Table IV-2a. Occupational handler exposure to carbaryl by the dermal and inhalation routes - short-term, seasonal, annual and lifetime estimates

| Exposure scenario                                                                   | STADD (mg/kg/day) <sup>a</sup> |            |        | SADD (mg/kg/day) <sup>b</sup> |            |        | AADD (mg/kg/day) <sup>c</sup> |            |         | LADD (mg/kg/day) <sup>d</sup> |            |         |
|-------------------------------------------------------------------------------------|--------------------------------|------------|--------|-------------------------------|------------|--------|-------------------------------|------------|---------|-------------------------------|------------|---------|
|                                                                                     | Dermal                         | Inhalation | Total  | Dermal                        | Inhalation | Total  | Dermal                        | Inhalation | Total   | Dermal                        | Inhalation | Total   |
| <b>Handlers: aerial applications (DPR, 2012: Tables 22 and 23)</b>                  |                                |            |        |                               |            |        |                               |            |         |                               |            |         |
| <u>Aerial (liquids)</u>                                                             |                                |            |        |                               |            |        |                               |            |         |                               |            |         |
| Mixer / loader                                                                      | 60.8                           | 0.0440     | 60.8   | 1.25                          | 0.000905   | 1.25   | 0.313                         | 0.000226   | 0.313   | 0.167                         | 0.000121   | 0.167   |
| Applicator                                                                          | 0.521                          | 0.00550    | 0.526  | 0.0107                        | 0.000113   | 0.0108 | 0.00268                       | 0.0000282  | 0.00270 | 0.00143                       | 0.0000150  | 0.00144 |
| <u>High-acre aerial (liquid)</u>                                                    |                                |            |        |                               |            |        |                               |            |         |                               |            |         |
| Mixer / loader                                                                      | 26.0                           | 0.0189     | 26.0   |                               |            |        |                               |            |         |                               |            |         |
| Applicator                                                                          | 0.223                          | 0.00236    | 0.226  |                               |            |        |                               |            |         |                               |            |         |
| Flagger                                                                             | 0.239                          | 0.00175    | 0.241  |                               |            |        |                               |            |         |                               |            |         |
| <u>High-acre aerial (granule)</u>                                                   |                                |            |        |                               |            |        |                               |            |         |                               |            |         |
| Loader                                                                              | 0.527                          | 0.0283     | 0.556  |                               |            |        |                               |            |         |                               |            |         |
| Applicator                                                                          | 0.0731                         | 0.116      | 0.189  |                               |            |        |                               |            |         |                               |            |         |
| Flagger                                                                             | 0.00994                        | 0.00151    | 0.0115 |                               |            |        |                               |            |         |                               |            |         |
| <b>Handlers: airblast and groundboom applications (DPR, 2012: Tables 24 and 25)</b> |                                |            |        |                               |            |        |                               |            |         |                               |            |         |
| <u>Airblast</u>                                                                     |                                |            |        |                               |            |        |                               |            |         |                               |            |         |
| Mixer / loader                                                                      | 6.94                           | 0.0503     | 6.99   | 1.56                          | 0.0113     | 1.57   | 0.781                         | 0.00566    | 0.787   | 0.417                         | 0.00302    | 0.420   |
| Applicator, citrus                                                                  | 0.591                          | 0.0122     | 0.604  | 0.133                         | 0.00275    | 0.136  | 0.0664                        | 0.00137    | 0.0678  | 0.0354                        | 0.000733   | 0.0362  |
| Applicator, 5-7.5 °                                                                 | 0.333                          | 0.0409     | 0.374  | 0.0650                        | 0.0117     | 0.0767 | 0.0163                        | 0.00292    | 0.0192  | 0.00867                       | 0.00156    | 0.0102  |
| Applicator, <5 °                                                                    | 4.01                           | 0.0302     | 4.04   | 0.961                         | 0.00722    | 0.968  | 0.160                         | 0.00120    | 0.161   | 0.0854                        | 0.000854   | 0.0860  |
| <u>Groundboom</u>                                                                   |                                |            |        |                               |            |        |                               |            |         |                               |            |         |
| Mixer / loader                                                                      | 2.31                           | 0.0168     | 2.33   | 0.417                         | 0.00302    | 0.420  | 0.104                         | 0.000754   | 0.105   | 0.0556                        | 0.000402   | 0.0560  |
| Applicator                                                                          | 0.136                          | 0.00942    | 0.146  | 0.0245                        | 0.00169    | 0.0262 | 0.00613                       | 0.000423   | 0.00655 | 0.00327                       | 0.000226   | 0.00350 |
| <u>High-acre groundboom</u>                                                         |                                |            |        |                               |            |        |                               |            |         |                               |            |         |
| Mixer / loader                                                                      | 5.78                           | 0.0419     | 5.82   | 0.573                         | 0.00415    | 0.577  | 0.143                         | 0.00104    | 0.144   | 0.0764                        | 0.000553   | 0.0770  |
| Applicator                                                                          | 0.341                          | 0.0235     | 0.365  | 0.0337                        | 0.00233    | 0.0361 | 0.00843                       | 0.000581   | 0.00901 | 0.00450                       | 0.000310   | 0.00481 |

| <b>Handlers: hand-held applications (DPR, 2012: Tables 26 and 27)</b>                                     |                  |                       |                  |                   |                       |                   |                     |                       |                     |                     |                       |                     |
|-----------------------------------------------------------------------------------------------------------|------------------|-----------------------|------------------|-------------------|-----------------------|-------------------|---------------------|-----------------------|---------------------|---------------------|-----------------------|---------------------|
| <u>Right-of-way</u><br>Mixer / loader<br>Applicator                                                       | 1.45<br>48.8     | 0.0105<br>0.0176      | 1.46<br>48.8     |                   |                       |                   |                     |                       |                     |                     |                       |                     |
| <u>Backpack sprayer</u><br>Mixer / loader / applicator                                                    | 5.34             | 0.00598               | 5.35             | 0.619             | 1.27x10 <sup>-4</sup> | 0.619             | 0.361               | 7.38x10 <sup>-5</sup> | 0.361               | 0.193               | 3.94x10 <sup>-5</sup> | 0.193               |
| <u>High-pressure handwand</u><br>Mixer / loader / applicator                                              | 41.3             | 1.26                  | 42.6             | 0.191             | 5.80x10 <sup>-3</sup> | 0.197             | 0.111               | 3.38x10 <sup>-3</sup> | 0.114               | 0.0593              | 1.80x10 <sup>-3</sup> | 0.0611              |
| <u>Low-pressure handwand</u><br>Mixer / loader / applicator                                               | 0.260            | 0.00176               | 0.262            | 0.0113            | 1.64x10 <sup>-4</sup> | 0.0115            | 0.00662             | 9.55x10 <sup>-5</sup> | 0.00671             | 0.00353             | 5.09x10 <sup>-5</sup> | 0.00358             |
| <u>Trigger spray applicator</u><br>Mixer / loader / applicator                                            | 0.000939         | 7.59x10 <sup>-6</sup> | 0.000946         | 0.0000921         | 2.16x10 <sup>-6</sup> | 0.0000943         | 0.0000537           | 1.26x10 <sup>-6</sup> | 0.0000550           | 0.0000286           | 6.73x10 <sup>-7</sup> | 0.0000293           |
| <u>Hose-end sprayer</u><br>Mixer / loader / applicator                                                    | 0.580            | 0.000854              | 0.581            | 0.00694           | 3.46x10 <sup>-5</sup> | 0.00697           | 0.00405             | 2.20x10 <sup>-5</sup> | 0.00407             | 0.00216             | 1.08x10 <sup>-5</sup> | 0.00217             |
| <b>Handlers: ground applications of carbaryl dust and granular products (DPR, 2012: Tables 28 and 29)</b> |                  |                       |                  |                   |                       |                   |                     |                       |                     |                     |                       |                     |
| <u>Broadcast spreader</u><br>Mixer / loader<br>Applicator                                                 | 0.0576<br>0.0125 | 0.0251<br>0.00167     | 0.0827<br>0.0141 | 0.0104<br>0.00224 | 0.00454<br>0.000299   | 0.0149<br>0.00254 | 0.00259<br>0.000560 | 0.00113<br>0.0000749  | 0.00372<br>0.000635 | 0.00138<br>0.000299 | 0.000605<br>0.0000399 | 0.00199<br>0.000339 |
| <u>High-acre broadcast spreader</u><br>Mixer / loader<br>Applicator                                       | 0.144<br>0.0312  | 0.0629<br>0.00417     | 0.207<br>0.0353  | 0.0143<br>0.00308 | 0.00624<br>0.000412   | 0.0205<br>0.00349 | 0.00356<br>0.000770 | 0.00156<br>0.000103   | 0.00512<br>0.000873 | 0.00190<br>0.000411 | 0.000832<br>0.0000549 | 0.00273<br>0.000466 |
| <u>Push-type spreader</u><br>Loader / applicator                                                          | 0.259            | 0.00188               | 0.261            | 0.0219            | 0.000251              | 0.0222            | 0.0128              | 0.000147              | 0.0129              | 0.00682             | 0.0000782             | 0.00690             |
| <u>Dust applicator</u><br>Loader / applicator                                                             | 0.136            | 0.0101                | 0.146            | 0.0253            | 0.00163               | 0.0269            | 0.0148              | 0.000950              | 0.0157              | 0.00787             | 0.000507              | 0.00838             |

*Note:* For details concerning the calculations of the values in this table, including the source of the raw data and the scenario-dependent requirements for personal protective equipment, see the indicated tables and text in DPR (2011). SADDs, AADDs and LADDs were not calculated for high-acre liquid and granular applications. According to DPR (2011), only short-term estimates were needed for these scenarios.

<sup>a</sup> STADD [i.e., short-term absorbed daily dosage] = [(short-term exposure) x (absorption) x (acres treated/day) x (application rate)] ÷ 70 kg bw. Calculation assumptions include: dermal absorption = 70%; inhalation rate = 16.7 L/min; inhalation absorption = 100%.

<sup>b</sup> SADD [i.e., seasonal average daily dosage] = [(long-term exposure) x (absorption) x (acres treated/day) x (application rate)] ÷ 70 kg bw. SADD is a 90% upper confidence estimate calculated from the long-term exposure rates provided in DPR (2011)

<sup>c</sup> AADD [i.e., annual average daily dosage] = [SADD x (annual use in months/yr)] / (12 months/yr). Annual exposure estimate was based on high-use period (DPR, 2012).

<sup>d</sup> LADD [i.e., lifetime average daily dosage] = [AADD x (40 yr of work per lifetime)] ÷ (75 yr/lifetime).

<sup>e</sup> Open-cab airblast applying at rates of 5 - 7.5 lb/acres; applicator must wear coverall and chemical-resistant head gear in addition to other handler requirements.

<sup>f</sup> Open-cab airblast applications at rates less than 5 lb/acre. There is no assumption of *additional* personal protective equipment.

Table IV-2b. Occupational reentry exposure to carbaryl by the dermal route - short-term, seasonal, annual and lifetime estimates

| Exposure scenario                | STADD (mg/kg/day) <sup>a</sup> | SADD (mg/kg/day) <sup>b</sup> | AADD (mg/kg/day) <sup>c</sup> | LADD (mg/kg/day) <sup>d</sup> |
|----------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Apple hand thinning              | 3.41                           | 2.07                          | 0.517                         | 0.276                         |
| Asparagus hand harvesting        | 0.363                          |                               |                               |                               |
| Beans scouting                   | 0.727                          |                               |                               |                               |
| Blackberry pruning               | 3.23                           |                               |                               |                               |
| Cabbage scouting                 | 1.38                           |                               |                               |                               |
| Citrus pruning                   | 6.84                           | 4.56                          | 2.66                          | 1.42                          |
| Corn detasseling                 | 4.52                           | 2.73                          | 0.228                         | 0.121                         |
| Cucumber scouting                | 0.421                          | 0.00024                       | 0.00010                       | 0.000053                      |
| Grape leaf pulling               | 1.74                           | 0.319                         | 0.0532                        | 0.0284                        |
| Lettuce scouting                 | 1.14                           | 0.690                         | 0.115                         | 0.0613                        |
| Olive pruning                    | 0.193                          | 0.0694                        | 0.00578                       | 0.00308                       |
| Ornamental plant hand harvesting | 0.131                          | 0.0854                        | 0.0427                        | 0.0228                        |
| Potato scouting                  | 0.970                          | 0.133                         | 0.0555                        | 0.0296                        |
| Strawberry scouting              | 0.129                          | 0.0355                        | 0.0178                        | 0.00947                       |
| Tobacco hand harvesting          | 0.757                          |                               |                               |                               |
| Tomato staking / tying           | 0.363                          | 0.0888                        | 0.0222                        | 0.0118                        |
| Turf maintenance                 | 2.74                           |                               |                               |                               |

Note: These values were taken from DPR's exposure assessment document on carbaryl, Tables 31 and 32 (DPR, 2012). Seasonal, annual and lifetime exposures to carbaryl were not expected for workers reentering treated asparagus, bean, blackberry, cabbage or tobacco crops. In

addition, such exposures were not expected for turf maintenance reentry workers. Fieldworkers were not required to wear personal protective equipment; consequently, these exposure estimates did not assume the workers were wearing such equipment.

<sup>a</sup> STADD [i.e., short-term absorbed daily dosage] = [DA x DFR x TC x ED] ÷ body weight (70 kg)

DA, dermal absorption rate of 70%

DFR, dislodgeable foliar residue in  $\mu\text{g}/\text{cm}^2$  at the expiration of the restricted entry or pre-harvest interval (DPR, 2012: Table 31)

TC, transfer coefficient in  $\text{cm}^2/\text{hr}$  (DPR, 2012: Table 31)

ED, exposure duration in hr/day

<sup>b</sup> SADD [i.e., seasonal average daily dosage] = [DA x ltDFR x TC x ED] ÷ body weight (70 kg)

ltDFR, long-term dislodgeable foliar residue (DPR, 2012: Table 32)

<sup>c</sup> AADD [i.e., annual average daily dosage] = [SADD x (months of application in year)] ÷ 12 months

<sup>d</sup> LADD (i.e., lifetime average daily dosage) = [LADD x (40 yr labor)] ÷ 75 yr lifespan

### 3. Residential handler and residential reentry exposure

Residential handler exposure estimates, which include a series of applications involving handwands, backpack, hose-end and trigger sprayers, shaker cans and push-type spreaders, appear in Table IV-3. Residential post-application (reentry) exposures for adults and toddlers reentering turf that had recently been treated with carbaryl also appear that Table. The possibility of additional exposure resulting from hand-to-mouth transfer in toddlers upon reentry to treated turf was considered by WH&S to be negligible. Seasonal, annual and lifetime exposures were not anticipated.

Table IV-3. Residential handler and residential turf reentry exposure to carbaryl by the dermal and inhalation routes - short-term estimates

| Exposure scenario                                                           | STADD (mg/kg/day) <sup>a</sup> |                     |          |
|-----------------------------------------------------------------------------|--------------------------------|---------------------|----------|
|                                                                             | Dermal                         | Inhalation          | Total    |
| <b>Residential handlers (DPR, 2012: Table 33)</b>                           |                                |                     |          |
| <b>Backpack mixer / loader / applicator</b>                                 | 0.163                          | 0.000182            | 0.163    |
| <b>Low pressure handwand mixer / loader / applicator</b>                    | 0.00792                        | 0.0000535           | 0.00794  |
| <b>Trigger spray applicator</b>                                             | 0.000939                       | 0.00000759          | 0.000946 |
| <b>Hose-end mixer / loader / applicator</b>                                 | 0.00707                        | 0.0000104           | 0.00708  |
| <b>Dust loader / applicator</b>                                             | 0.0259                         | 0.000188            | 0.0261   |
| <b>Push-type spreader loader / applicator</b>                               | 0.136                          | 0.0101              | 0.146    |
| <b>Residential reentry onto carbaryl-treated turf (DPR, 2012: Table 34)</b> |                                |                     |          |
| <b>Adults <sup>b</sup></b>                                                  | 2.58                           | / / / / / / / / / / | 2.58     |
| <b>Toddlers <sup>b</sup></b>                                                | 4.33                           | / / / / / / / / / / | 4.33     |

Note: For further details concerning the calculations of the values in this table, including the source of the raw data and the personal protective equipment requirements, see Table 33 and text in DPR (2012). Only short-term uses were anticipated for residential handler scenarios; consequently, there were no SADD, AADD or LADD estimates.

<sup>a</sup>  $STADD_{handler} [i.e., \text{short-term absorbed daily dosage}] = [(short\text{-term exposure}) \times (absorption) \times (acres\ treated/day) \times (application\ rate)] \div 70\ kg\ bw$ . Calculation assumptions include: dermal absorption = 70%; inhalation rate = 16.7 L/min; inhalation absorption = 100%.

$$STADD_{reentry} = (95^{th}\ \text{percentile exposure rate in } \mu\text{g/kg/hr}) \times (2\ \text{hr/day}) \times (70\% \text{ dermal absorption})$$

<sup>b</sup> Significant inhalation exposure upon reentry to turf previously treated with carbaryl was considered to be unlikely. Two hour dermal exposure sessions were used as a default. The adult body weight of 69.4 kg was the mean determined in an exposure monitoring study cited in DPR (2011). The toddler body weight of 15 kg was from the same study.

#### 4. Toddler exposures from hand-to-mouth, object-to-mouth and soil ingestion behaviors

Oral exposures in toddlers exposed through hand-to-mouth, object-to-mouth and soil ingestion behaviors were estimated from environmental residues and from assumptions detailed in DPR (2011). These estimates are contained in Table IV-4.

Table IV-4. Oral carbaryl exposure in toddlers through hand-to-mouth, object-to-mouth and soil ingestion behaviors

| Exposure scenario        | STADD (mg/kg/day)     |
|--------------------------|-----------------------|
| Hand-to-mouth transfer   | 0.00250 <sup>a</sup>  |
| Object-to-mouth transfer | 0.00162 <sup>b</sup>  |
| Soil ingestion           | 0.000229 <sup>c</sup> |

<sup>a</sup> For hand-to-mouth transfer,  $STADD = [(TTR) \times (HSA) \times (\text{events/hr}) \times (\% \text{ transferable}) \times (2 \text{ hr/day})] \div 15 \text{ kg}$   
TTR, turf transferable residue = 0.939  $\mu\text{g}/\text{cm}^2$   
HSA, hand surface area contacting mouth = 20  $\text{cm}^2$   
events/hr, rate of hand-to-mouth contact = 20 events/hr  
% transferable, percent of residues transferable from hand to mouth with each contact = 50% (or 0.5)

<sup>b</sup> For object-to-mouth transfer,  $STADD = [(TTR) \times (OSA) \times (\% \text{ transferable})] \div 15 \text{ kg}$   
TTR, turf transferable residue = 0.939  $\mu\text{g}/\text{cm}^2$   
OSA, object surface area = 25  $\text{cm}^2$   
% transferable, percent of residues transferable from hand to mouth with each contact = 100% (or 1.0)

<sup>c</sup> For soil ingestion,  
 $STADD = [(8.28 \text{ lb ai/acre}) \times (0.1 \text{ g/day}) \times (1 \text{ cm}) \times (0.67 \text{ cm}^3/\text{g}) \times (4.54 \times 10^8 \text{ } \mu\text{g}/\text{lb}) \times (2.47 \times 10^{-8} \text{ acre}/\text{cm}^2)] \div 15 \text{ kg}$   
8.28 lb ai/acre = peak turf application rate  
0.1 g/day = amount of soil ingested / day  
0.67  $\text{cm}^3/\text{g}$  = bulk soil density

## 5. Swimmer exposure

The potential for swimmer exposure was estimated because carbaryl was detected in California surface waters. The values provided by WH&S take into account both dermal absorption and oral ingestion in swimmers. As such, they are expressed as aggregate internal doses (Table IV-5). The default assumptions underlying these estimates are contained in DPR (2011).

Table IV-5. Exposure of swimmers to carbaryl in surface water

|              | STADD <sup>a</sup><br>mg/kg/day | SADD <sup>b</sup><br>mg/kg/day | AADD <sup>c</sup><br>mg/kg/day |
|--------------|---------------------------------|--------------------------------|--------------------------------|
| Adult        | 0.000043                        | 0.00000016                     | 0.000000000044                 |
| Child (6 yr) | 0.00011                         | 0.00000070                     | 0.00000000019                  |

<sup>a</sup> Estimations of STADD, SADD and AADD were executed as described in DPR (2011). The short-term carbaryl concentration was assumed to be 6.94 µg/L with an exposure duration of 5 hr. The seasonal and annual carbaryl concentrations were assumed to be 0.0001 µg/L with an exposure duration of 2.3 hr/day for children and 1.3 hr/day for adults.

## 6. Bystander (application site) exposure

The potential for inhalation exposure was estimated for bystanders situated near fields or orchards during or directly after specific agricultural applications. Because a carbaryl-specific application site study was unavailable, exposure estimates for those scenarios were derived from a surrogate study using methyl parathion. The maximum application rate for methyl parathion was 2 lb/acre, while that of carbaryl was 12 lb/acre. Thus the values from the methyl parathion study were multiplied by a factor of six to obtain carbaryl estimates, which are summarized in Table IV-6 (DPR, 2012).

In addition to exposures resulting from agricultural applications, bystanders also may be exposed during and after urban and suburban applications for the purpose of public pest control. Estimates for those scenarios were obtained from DPR carbaryl monitoring studies conducted at various sites in California. These are also summarized in Table xxxf.

Table IV-6. Carbaryl exposure to bystanders resulting from agricultural and public pest control applications

| <b>Absorbed dose</b>                                                          |                    |
|-------------------------------------------------------------------------------|--------------------|
| <b>Bystander exposure, agricultural applications (DPR, 2012: Table 36)</b>    |                    |
| <b>1-hr absorbed dose (heavy activity)</b>                                    |                    |
| <b>Infant</b>                                                                 | 0.0110 mg/kg/hr    |
| <b>Adult</b>                                                                  | 0.00198 mg/kg/hr   |
| <b>Short-term absorbed daily dosage (STADD)</b>                               |                    |
| <b>Infant</b>                                                                 | 0.0192 mg/kg/day   |
| <b>Adult</b>                                                                  | 0.00910 mg/kg/day  |
| <b>Seasonal absorbed daily dosage (SADD)</b>                                  |                    |
| <b>Infant</b>                                                                 | 0.00469 mg/kg/day  |
| <b>Adult</b>                                                                  | 0.00223 mg/kg/day  |
| <b>Annual absorbed daily dosage (AADD)</b>                                    |                    |
| <b>Infant</b>                                                                 | 0.000391 mg/kg/day |
| <b>Adult</b>                                                                  | 0.000186 mg/kg/day |
| <b>Lifetime absorbed daily dosage (LADD)</b>                                  |                    |
| <b>Infant</b>                                                                 | n/a                |
| <b>Adult</b>                                                                  | 0.000186 mg/kg/day |
| <b>Bystander exposure, public pest control programs (DPR, 2012: Table 37)</b> |                    |
| <b>1-hr absorbed dose (heavy activity)</b>                                    |                    |
| <b>Infant</b>                                                                 | 0.0030mg/kg/hr     |
| <b>Adult</b>                                                                  | 0.00054 mg/kg/hr   |
| <b>Short-term absorbed daily dosage (STADD)</b>                               |                    |
| <b>Infant</b>                                                                 | 0.00015 mg/kg/day  |
| <b>Adult</b>                                                                  | 0.000027 mg/kg/day |

Note: Assumptions underlying the calculations in this table are found in DPR (2011), Tables 36 and 37.

## **7. Ambient exposure**

Exposure to carbaryl in the air that is not associated with specific applications may occur. As detailed in DPR (2011), ambient exposure was not expected to exceed application site exposure and was not estimated in the WH&S document.

## C. RISK CHARACTERIZATION

### 1. Introduction

The potential for non-oncogenic health effects resulting from carbaryl exposure was expressed as the margin of exposure (MOE). The MOE is the ratio of the critical NOEL or LED value, as derived from the definitive acute, subchronic or chronic studies, divided by the estimated exposure.

$$\text{Margin of Exposure (MOE)} = \frac{\text{NOEL or LED (mg/kg)}}{\text{Exposure dose (mg/kg)}}$$

In general, MOEs of 10 or more are considered protective of human health if the relevant adverse effects were observed in human experimental toxicity studies. This reflects the default assumption that a 10-fold range of sensitivity exists within the human population. MOEs of 100 are generally considered to be protective of human health if the relevant adverse effects were observed in experimental animal studies. This reflects the default assumptions that (1) humans are 10-fold more sensitive than animals and (2) that a 10-fold range of sensitivity exists within the human population. All of the critical endpoints used in this report were derived from animal studies.

As noted in the accompanying exposure assessment document (DPR, 2012) and summarized above in section IV.B., the exposure estimates for carbaryl were derived from four sources: (1) surrogate data in the Pesticide Handlers Exposure Database (PHED), which predicts both dermal and inhalation exposure to handlers, (2) reentry scenarios involving dermal exposure to fieldworkers through contact with dislodgeable foliar residues, (3) air monitoring studies designed to estimate bystander and ambient exposures by the inhalation route, and (4) residue studies on food items. The following sections provide the MOE values generated by these exposure scenarios.

Oncogenic risk under occupational, bystander and dietary scenarios was assessed by estimating the increased cancer incidence resulting from the anticipated lifetime average daily dose. This was calculated as the product of the potency value-- $1.01 \times 10^{-2} \text{ mg/kg/day}^{-1}$  based on the increased incidence of hemangiomas and hemangiosarcomas after dietary exposure in mice (Hamada, 1993b)--and the lifetime average daily dose (LADD) in mg/kg/day. The resultant unitless value represents the increased risk to population exposed at that particular LADD. Risk values less than  $10^{-6}$  (*i.e.*, <1 excess cancer per one million individuals) are considered negligible.

### 2. Occupational handler and occupational reentry exposure risks

MOEs and oncogenic risk values for occupational handler exposure scenarios appear in Table IV-7a. Occupational reentry exposure risks appear in Table IV-7b. Many occupational handler and reentry scenarios yielded MOEs of less than 100 by the dermal and inhalation routes, with some less than 1. Oncogenic risk commonly exceeded  $10^{-6}$  for both exposure routes. For example, among occupational handlers, mixer / loaders appeared to be at greatest risk, comprising a large number of those exhibiting acute MOEs less than 1, as well as those with seasonal and annual MOEs less than 100 and oncogenic risk exceeding  $10^{-3}$ .

These tables also provide MOEs for aggregate exposures, which were calculated using the "hazard index" approach (footnote c, Table xxxg). Thus in Table xxxg, "aggregate" risk refers either to dermal + inhalation risk or dermal + inhalation + dietary risk (the latter in parentheses). In Table xxxh, the parenthetic values represent dermal + dietary risk (no inhalation exposure was expected for reentry tasks). The aggregate risk calculations assumed acute and chronic dietary MOEs of 228 and 1973, respectively, for the working population <sup>16</sup>. MOEs dipped below 100 for four handler scenarios in which the individual contributing MOEs were greater than 100: (1) short-term groundboom applicators (dermal + inhalation + dietary); (2) short-term high-acre broadcast spreader applicators (dermal + inhalation + dietary); (3) seasonal airblast citrus applicators (dermal + inhalation); and (4) annual high-pressure handwand mixer / loader / applicators (dermal + inhalation). These aggregate MOEs are underlined in Table xxxh in order to direct the reader's attention to scenarios in which aggregation may indicate a need to consider mitigation. Many other combined exposure MOEs were also below 100, but in each of the latter cases at least one of the individual contributing MOEs was already below 100.

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<sup>16</sup> The acute dietary MOE pertained to adults 16-70 yr. It represented the 99.9th percentile exposure level as derived by Monte Carlo simulation using the Dietary Exposure Evaluation Model (DEEM-FCID; DPR, 2012). The chronic dietary MOE pertained to adults 20-49 yr. The Dietary Exposure Evaluation Model did not provide the adult 16-70 yr population for chronic exposure evaluation.

Table IV-7a. Occupational handler risks from carbaryl exposure by the dermal and inhalation routes - short-term, seasonal, annual and lifetime exposure scenarios

| Exposure scenario                                                                   | Short-term MOE      |                         |                        | Seasonal MOE        |                         |                        | Annual MOE          |                         |                        | Oncogenic risk <sup>d</sup> |                       |                       |
|-------------------------------------------------------------------------------------|---------------------|-------------------------|------------------------|---------------------|-------------------------|------------------------|---------------------|-------------------------|------------------------|-----------------------------|-----------------------|-----------------------|
|                                                                                     | Dermal <sup>a</sup> | Inhalation <sup>b</sup> | Aggregate <sup>c</sup> | Dermal <sup>a</sup> | Inhalation <sup>b</sup> | Aggregate <sup>c</sup> | Dermal <sup>a</sup> | Inhalation <sup>b</sup> | Aggregate <sup>c</sup> | Dermal                      | Inhalation            | Aggregate             |
| <b>Handlers: aerial applications (DPR, 2012: Tables 22 and 23)</b>                  |                     |                         |                        |                     |                         |                        |                     |                         |                        |                             |                       |                       |
| <b>Aerial (liquids)</b>                                                             |                     |                         |                        |                     |                         |                        |                     |                         |                        |                             |                       |                       |
| Mixer / loader                                                                      | 0.23                | 23                      | 0.23 (0.23)            | 11                  | 552                     | 11                     | 45                  | 2212                    | 44 (43)                | 1.69x10 <sup>-3</sup>       | 1.22x10 <sup>-6</sup> | 1.69x10 <sup>-3</sup> |
| Applicator                                                                          | 29                  | 182                     | 25 (23)                | 1308                | 4425                    | 1010                   | 5224                | 17,730                  | 4035 (1325)            | 1.44x10 <sup>-5</sup>       | 1.52x10 <sup>-7</sup> | 1.45x10 <sup>-5</sup> |
| <b>High-acre aerial (liquid)</b>                                                    |                     |                         |                        | /                   |                         |                        |                     |                         |                        |                             |                       |                       |
| Mixer / loader                                                                      | 0.54                | 53                      | 0.53 (1)               | /                   |                         |                        |                     |                         |                        |                             |                       |                       |
| Applicator                                                                          | 63                  | 424                     | 55 (44)                | /                   |                         |                        |                     |                         |                        |                             |                       |                       |
| Flagger                                                                             | 59                  | 571                     | 53 (43)                | /                   |                         |                        |                     |                         |                        |                             |                       |                       |
| <b>High-acre aerial (granule)</b>                                                   |                     |                         |                        | /                   |                         |                        |                     |                         |                        |                             |                       |                       |
| Loader                                                                              | 27                  | 35                      | 15 (15)                | /                   |                         |                        |                     |                         |                        |                             |                       |                       |
| Applicator                                                                          | 192                 | 9                       | 8 (8)                  | /                   |                         |                        |                     |                         |                        |                             |                       |                       |
| Flagger                                                                             | 1408                | 662                     | 450 (151)              | /                   |                         |                        |                     |                         |                        |                             |                       |                       |
| <b>Handlers: airblast and groundboom applications (DPR, 2012: Tables 24 and 25)</b> |                     |                         |                        |                     |                         |                        |                     |                         |                        |                             |                       |                       |
| <b>Airblast</b>                                                                     |                     |                         |                        |                     |                         |                        |                     |                         |                        |                             |                       |                       |
| Mixer / loader                                                                      | 2                   | 20                      | 2 (2)                  | 9                   | 44                      | 7                      | 18                  | 88                      | 16 (15)                | 4.20x10 <sup>-3</sup>       | 3.05x10 <sup>-5</sup> | 4.24x10 <sup>-3</sup> |
| Applicator, citrus                                                                  | 24                  | 82                      | 18 (17)                | 105                 | 182                     | 67                     | 211                 | 365                     | 134 (125)              | 3.58x10 <sup>-4</sup>       | 7.40x10 <sup>-5</sup> | 3.66x10 <sup>-4</sup> |
| Applicator, 5-7.5 lb                                                                | 42                  | 24                      | 15 (14)                | 215                 | 43                      | 36                     | 859                 | 171                     | 143 (133)              | 8.76x10 <sup>-5</sup>       | 1.58x10 <sup>-5</sup> | 1.03x10 <sup>-4</sup> |
| Applicator, <5 lb                                                                   | 3                   | 33                      | 3 (3)                  | 16                  | 69                      | 13                     | 88                  | 417                     | 73 (70)                | 8.63x10 <sup>-4</sup>       | 8.63x10 <sup>-6</sup> | 8.69x10 <sup>-4</sup> |
| <b>Groundboom</b>                                                                   |                     |                         |                        |                     |                         |                        |                     |                         |                        |                             |                       |                       |
| Mixer / loader                                                                      | 6                   | 493                     | 6 (6)                  | 34                  | 166                     | 28                     | 135                 | 663                     | 112 (106)              | 5.62x10 <sup>-4</sup>       | 4.06x10 <sup>-5</sup> | 5.66x10 <sup>-4</sup> |
| Applicator                                                                          | 103                 | 13,077                  | 102 (71)               | 561.43              | 295.86                  | 193.76                 | 2284                | 1182                    | 779 (558)              | 3.30x10 <sup>-5</sup>       | 2.28x10 <sup>-6</sup> | 3.54x10 <sup>-5</sup> |
| <b>High-acre groundboom</b>                                                         |                     |                         |                        |                     |                         |                        |                     |                         |                        |                             |                       |                       |
| Mixer / loader                                                                      | 2                   | 24                      | 2 (2)                  | 24                  | 120                     | 20                     | 98                  | 481                     | 81 (78)                | 7.72x10 <sup>-4</sup>       | 5.59x10 <sup>-6</sup> | 7.78x10 <sup>-4</sup> |
| Applicator                                                                          | 41                  | 43                      | 21 (19)                | 415                 | 215                     | 141                    | 1661                | 861                     | 567 (440)              | 4.55x10 <sup>-5</sup>       | 3.13x10 <sup>-6</sup> | 4.86x10 <sup>-5</sup> |

| <b>Handlers: hand-held applications (DPR, 2012: Tables 26 and 27)</b>                                     |        |         |              |         |         |        |         |         |               |                       |                       |                       |
|-----------------------------------------------------------------------------------------------------------|--------|---------|--------------|---------|---------|--------|---------|---------|---------------|-----------------------|-----------------------|-----------------------|
| <b>Right-of-way</b><br>Mixer / loader                                                                     | 10     | 95      | 9 (9)        |         |         |        |         |         |               |                       |                       |                       |
| Applicator                                                                                                | 0.29   | 57      | 0.29 (0.29)  |         |         |        |         |         |               |                       |                       |                       |
| <b>Backpack sprayer</b><br>Mixer / loader / applicator                                                    | 3      | 167     | 3 (3)        | 23      | 3937    | 61     | 39      | 6775    | 39 (38)       | $1.95 \times 10^{-3}$ | $3.98 \times 10^{-7}$ | $1.95 \times 10^{-3}$ |
| <b>High-pressure handwand</b><br>Mixer / loader / applicator                                              | 0.34   | 0.79    | 0.24 (0.24)  | 73      | 86      | 40     | 126     | 148     | 68 (66)       | $5.99 \times 10^{-4}$ | $1.82 \times 10^{-5}$ | $6.17 \times 10^{-4}$ |
| <b>Low-pressure handwand</b><br>Mixer / loader / applicator                                               | 54     | 568     | 49 (41)      | 1239    | 3049    | 881    | 2258    | 5236    | 1578 (877)    | $3.57 \times 10^{-5}$ | $5.14 \times 10^{-7}$ | $3.62 \times 10^{-5}$ |
| <b>Trigger spray applicator</b><br>Mixer / loader / applicator                                            | 14,909 | 131,752 | 13,394 (224) | 152,009 | 231,481 | 91,755 | 260,708 | 396,825 | 157,339(1949) | $2.89 \times 10^{-7}$ | $6.80 \times 10^{-9}$ | $2.96 \times 10^{-7}$ |
| <b>Hose-end sprayer</b><br>Mixer / loader / applicator                                                    | 24     | 1171    | 24 (21)      | 2017    | 14,451  | 1770   | 3457    | 22,727  | 3001 (1190)   | $2.18 \times 10^{-5}$ | $1.09 \times 10^{-7}$ | $2.19 \times 10^{-5}$ |
| <b>Handlers: ground applications of carbaryl dust and granular products (DPR, 2012: Tables 28 and 29)</b> |        |         |              |         |         |        |         |         |               |                       |                       |                       |
| <b>Broadcast spreader</b><br>Mixer / loader                                                               | 243    | 40      | 34 (30)      | 1346    | 110     | 102    | 5405    | 442     | 409 (338)     | $1.39 \times 10^{-5}$ | $6.11 \times 10^{-6}$ | $2.01 \times 10^{-5}$ |
| Applicator                                                                                                | 1120   | 599     | 390 (144)    | 6250    | 1672    | 1319   | 25,000  | 6676    | 5269 (1435)   | $3.02 \times 10^{-6}$ | $4.03 \times 10^{-7}$ | $3.42 \times 10^{-6}$ |
| <b>High-acre broadcast spreader</b><br>Mixer / loader                                                     | 97     | 16      | 14 (13)      | 979     | 80      | 74     | 3933    | 321     | 296 (258)     | $1.92 \times 10^{-5}$ | $8.40 \times 10^{-6}$ | $2.76 \times 10^{-5}$ |
| Applicator                                                                                                | 449    | 240     | 156 (93)     | 4545    | 1214    | 956    | 18,182  | 4854    | 3831(1302)    | $4.15 \times 10^{-6}$ | $5.54 \times 10^{-7}$ | $4.71 \times 10^{-6}$ |
| <b>Push-type spreader</b><br>Loader / applicator                                                          | 54     | 532     | 49 (40)      | 639     | 1992    | 484    | 1094    | 3401    | 828 (583)     | $6.89 \times 10^{-5}$ | $7.90 \times 10^{-9}$ | $6.97 \times 10^{-5}$ |
| <b>Dust applicator</b><br>Loader / applicator                                                             | 103    | 99      | 50 (41)      | 553     | 307     | 197    | 946     | 526     | 338 (289)     | $7.95 \times 10^{-5}$ | $5.12 \times 10^{-6}$ | $8.46 \times 10^{-5}$ |

*Note:* The exposure values appear above in Table IV-2a. They were taken from DPR's exposure assessment document on carbaryl, Tables 23-30 (DPR, 2012). With the exception of MOEs of less than 1, MOEs were rounded to the nearest integer. This was not done to indicate precision, which was undoubtedly less than what is implied by the apparent exactitude expressed here, but to clarify for the reader how the calculations were made.

<sup>a</sup> Because dermal absorption under all scenarios was 70% and the critical acute, subchronic and chronic dermal NOELs were determined by route specific (i.e., dermal) rat study, those NOELs were multiplied by 0.7 in order to obtain an absorbed NOEL useful for the calculations in this table. Thus the acute, subchronic and chronic dermal NOEL = 20 mg/kg x 0.7 = 14 mg/kg (Austin, 2002a). However, for oncogenic risk no adjustment for dermal absorption was made since the potency value came from an oral study.

<sup>b</sup> Acute inhalation NOEL = 1 mg/kg (acute oral study of Robinson and Broxup, 1997). Subchronic and chronic inhalation LED<sub>10</sub> = 0.5 mg/kg/day (chronic oral study of Hamada, 1987).

<sup>c</sup> The aggregate (multi-route) non-oncogenic risk for each exposure length--acute, subchronic and chronic--were calculated using the "hazard index", which was the reciprocal of the sum of the reciprocals of the dermal, inhalation and, when applicable, dietary MOE values. Values in parentheses represent the combined acute or chronic risk for dermal, inhalation *and* dietary exposure. The combined acute MOE assumed a Monte Carlo-derived, 99.9% percentile dietary acute MOE of 228 for working adults (DPR, 2010), based on the acute oral NOEL of 1 mg/kg. The combined chronic MOE assumed a chronic dietary MOE of 1973 for adults, 20-49 years old (DPR, 2010), based on the chronic oral NOEL of 0.5 mg/kg/day. Aggregate MOEs less than 100 in which the contributing MOEs were more than 100 are underlined.

<sup>d</sup> Oncogenic risk was calculated as the product of the potency value in mg/kg/day<sup>-1</sup> and the lifetime average daily dose in mg/kg/day. As such, it is a unitless value. There was no need to incorporate a dermal absorption factor since the oncogenic potency was derived from an oral study. Thus for aerial liquid exposure to mixer/loaders by the dermal route:  $(1.01 \times 10^{-2} \text{ mg/kg/day}^{-1}) (0.167 \text{ mg/kg/day}) = 1.69 \times 10^{-3}$ . Aggregate oncogenic risk was the product of the aggregate (i.e., dermal + inhalation) lifetime exposure found in the exposure assessment at the oncogenic potency. Dietary risk was not added to the aggregate total since at  $2.90 \times 10^{-6}$ , it already exceeded  $10^{-6}$  for the adult population (DPR, 2010).

Table IV-7b. MOEs and oncogenic risk values for occupational reentry carbaryl dermal exposure scenarios - short-term, seasonal, annual and lifetime estimates

| Exposure scenario                | MOE - short-term <sup>a</sup> | MOE - seasonal <sup>a</sup> | MOE - annual <sup>a</sup> | Oncogenic risk <sup>b</sup> |
|----------------------------------|-------------------------------|-----------------------------|---------------------------|-----------------------------|
| Apple hand thinning              | 4 (4)                         | 7                           | 27 (27)                   | 2.79x10 <sup>-3</sup>       |
| Asparagus hand harvesting        | 4 (4)                         |                             |                           |                             |
| Beans scouting                   | 19 (18)                       |                             |                           |                             |
| Blackberry pruning               | 4 (4)                         |                             |                           |                             |
| Cabbage scouting                 | 10 (10)                       |                             |                           |                             |
| Citrus pruning                   | 2 (2)                         | 3                           | 5 (5)                     | 1.43x10 <sup>-2</sup>       |
| Corn detasseling                 | 3 (3)                         | 5                           | 61 (59)                   | 1.22x10 <sup>-3</sup>       |
| Cucumber scouting                | 33 (29)                       | 58,333                      | 140,000 (1946)            | 5.35x10 <sup>-7</sup>       |
| Grape leaf pulling               | 8 (8)                         | 44                          | 263 (232)                 | 2.87x10 <sup>-4</sup>       |
| Lettuce scouting                 | 12 (11)                       | 20                          | 122 (115)                 | 6.19x10 <sup>-4</sup>       |
| Olive pruning                    | 73 (55)                       | 202                         | 24,221 (1824)             | 3.11x10 <sup>-7</sup>       |
| Ornamental plant hand harvesting | 107 (73)                      | 164                         | 328 (281)                 | 2.30x10 <sup>-4</sup>       |
| Potato scouting                  | 14 (13)                       | 105                         | 252 (223)                 | 2.99x10 <sup>-4</sup>       |
| Strawberry scouting              | 109 (74)                      | 394                         | 787 (562)                 | 9.56x10 <sup>-5</sup>       |
| Tobacco hand harvesting          | 18 (17)                       |                             |                           |                             |
| Tomato staking / tying           | 39 (33)                       | 158                         | 631 (478)                 | 1.19x10 <sup>-4</sup>       |
| Turf maintenance                 | 5 (5)                         |                             |                           |                             |

*Note:* The exposure values appear above in Table IV-2b. They were taken from DPR's exposure assessment document on carbaryl, Tables 30 and 31 (DPR, 2012). Combined risk from dermal and dietary exposures appear in parentheses (no combined value appears for seasonal risk because such a value was not calculated for dietary exposure). As with the handler scenarios, the combined acute MOE assumed a Monte Carlo-derived, 99.9% percentile dietary acute MOE of 228 for working adults (DPR, 2010), which was based on the acute oral NOEL of 1 mg/kg. The combined chronic MOE assumed a chronic dietary MOE of 1973 for adults, 20-49 years old (DPR, 2010), which was based on the chronic oral NOEL of 0.5 mg/kg/day. A combined dietary-dermal oncogenic risk calculation was considered unnecessary since the dietary risk value for the Western region,  $3.68 \times 10^{-6}$  (DPR, 2010), already exceeded the  $10^{-6}$  negligible risk standard. Seasonal, annual and lifetime exposures to carbaryl were not predicted for workers reentering treated asparagus, bean, blackberry, cabbage or tobacco fields. In addition, such exposures were not predicted for turf maintenance reentry workers. MOEs were rounded to the closest integer values (see Note to Table xxx.g).

<sup>a</sup> The critical dermal NOEL was 20 mg/kg (Austin, 2002b). After allowance for 70% dermal absorption (which made the NOEL 14 mg/kg), this value was used for acute, seasonal and annual dermal exposure scenarios.

<sup>b</sup> Oncogenic risk was calculated as the product of the potency value in  $\text{mg/kg/day}^{-1}$  and the lifetime average daily dose in mg/kg/day. As such, it is a unitless value. There was no need to incorporate a dermal absorption factor since the oncogenic potency was derived from an oral study. Thus for apple hand thinners:  $(1.01 \times 10^{-2} \text{ mg/kg/day}^{-1}) (0.276 \text{ mg/kg/day}) = 2.79 \times 10^{-3}$ .



<sup>d</sup> The combined non-oncogenic risk for acute exposures were calculated by the “hazard index” approach, which was equal to the inverse of the sum of the inverses of the contributory MOE values. Parenthetic values represent the risk when acute dietary exposure is also considered (see footnote "c", Tables xxxg.)

#### 4. Risks to toddlers exposures due to hand-to-mouth, object-to-mouth and soil ingestion behaviors

Short-term oral MOEs for residential toddlers resulting from hand-to-mouth, object-to-mouth and soil ingestion behaviors appear in Table IV-9. All MOEs equalled or exceeded 100. When combined with anticipated dietary exposures using the hazard index approach, all MOEs were below 100, mainly because the acute dietary MOE for children 1-2 yr was 92. Seasonal, annual and lifetime exposures were not anticipated for these scenarios.

Table IV-9. Risk from oral carbaryl exposure in toddlers through hand-to-mouth, object-to-mouth and soil ingestion behaviors

| Exposure scenario        | Short-term MOE <sup>a</sup> |
|--------------------------|-----------------------------|
| Hand-to-mouth transfer   | 400 (75)                    |
| Object-to-mouth transfer | 617 (80)                    |
| Soil ingestion           | 4367 (90)                   |

*Note:* The exposure values used to calculate MOEs in this table appear above in Table IV-4. MOEs were rounded to the closest integer values (see Note to Table IV-7a).

<sup>a</sup> Acute oral NOEL = 1mg/kg (acute oral study of Robinson and Broxup, 1997). Combined MOEs were created by adding exposure from dietary sources using the hazard index approach as described in Table xxxg above. The acute dietary MOE for children 1-2 yr at the 99.9th percentile using the Monte Carlo approach was 92 (DPR, 2010).

## 5. Risks to swimmers

Risks from short-term, seasonal and annual exposure of swimmers to carbaryl in surface waters are summarized in Table IV-10. The MOEs were calculated using aggregated dermal and oral exposure terms. The toxicity terms included the critical acute oral, subchronic and chronic NOELs. The oral toxicity values were chosen as a health-protective measure, since the critical oral values were lower than the dermal values. As all MOEs exceeded 100 by orders of magnitude (and were thus not indicative of a health risk), a combined assessment using dietary risks was not carried out, as the resultant values would simply reflect dietary risk.

Table IV-10. Short-term, seasonal and annual risk to swimmers exposed to carbaryl in surface water

|              | Short-term MOE <sup>a</sup> | Seasonal MOE <sup>b</sup> | Annual MOE <sup>b</sup> |
|--------------|-----------------------------|---------------------------|-------------------------|
| Adult        | 2.33x10 <sup>4</sup>        | 3.12x10 <sup>6</sup>      | 1.14x10 <sup>10</sup>   |
| Child (6 yr) | 9.09x10 <sup>3</sup>        | 7.14x10 <sup>5</sup>      | 2.63x10 <sup>9</sup>    |

Note: Swimmer exposures were expressed in Table IV-5 as an aggregate of oral and dermal exposures. The MOEs in this table were calculated using the lower (oral) of the acute NOELs. As they did not indicate a health risk, the analysis was not carried further. MOEs were rounded to the closest integer values (see Note to Table IV-7a).

<sup>a</sup> Acute oral NOEL / LED<sub>10</sub> = 1 mg/kg (acute oral study of Robinson and Broxup, 1997).

<sup>b</sup> Subchronic and chronic oral NOEL = 0.5 mg/kg/day (chronic oral study of Hamada, 1987).

## 6. Risks to bystanders

Bystander exposures by the inhalation route were expected to occur in individuals standing near applications, be they agricultural or public pest control operations. The risks, which included scenarios ranging from 1-hr MOEs based on heavy physical activity to lifetime oncogenicity estimates, appear in Table IV-11. 1-hr, short-term and seasonal MOEs were less than 100 for infant and adult bystanders with respect to agricultural applications. Combining risks from these scenarios with those resulting from dietary exposure to the same demographic resulted in MOEs less than 100 in several cases. In one case, adult short-term risk, MOEs of greater than 100 for both the inhalation and dietary routes, combined to generate an MOE of less than 100 (*i.e.*, 74). Oncogenic risk to adults was  $1.88 \times 10^{-6}$ .

Table IV-11. Non-oncogenic and oncogenic risks resulting from inhalational carbaryl exposure to bystanders – agricultural and public pest control applications

| Short-term MOE                                          |                       |
|---------------------------------------------------------|-----------------------|
| <b>Bystander exposure, agricultural applications</b>    |                       |
| <b>1-hr risk (heavy activity)<sup>a</sup></b>           |                       |
| Infant                                                  | 91 (46)               |
| Adult                                                   | 505 (157)             |
| <b>Short-term risk<sup>a</sup></b>                      |                       |
| Infant                                                  | 52 (33)               |
| Adult                                                   | 110 (74)              |
| <b>Seasonal risk<sup>b</sup></b>                        |                       |
| Infant                                                  | 107                   |
| Adult                                                   | 224                   |
| <b>Annual risk<sup>b</sup></b>                          |                       |
| Infant                                                  | 1279 (86)             |
| Adult                                                   | 2688 (210)            |
| <b>Lifetime oncogenic risk<sup>c</sup></b>              |                       |
| Infant                                                  | n/a                   |
| Adult                                                   | $1.88 \times 10^{-6}$ |
| <b>Bystander exposure, public pest control programs</b> |                       |
| <b>1-hr risk (heavy activity)<sup>a</sup></b>           |                       |
| Infant                                                  | 333 (72)              |
| Adult                                                   | 1852 (203)            |
| <b>Short-term risk<sup>a</sup></b>                      |                       |
| Infant                                                  | 6667 (91)             |
| Adult                                                   | 37,037 (227)          |

*Note:* The exposure assumptions underlying the calculations in this table are found in DPR (2011), Tables 36 and 37, and in the accompanying text. Combined MOEs were created by adding exposure from dietary sources using the hazard index approach as described in Table IV-10-7a above. The acute dietary MOE

for children 1-2 yr at the 99.9th percentile using the Monte Carlo approach was 92, while that for adults 16-70 years was 228 (DPR, 2010).

<sup>a</sup> Acute inhalation NOEL = 1 mg/kg (acute oral study of Robinson and Broxup, 1997).

<sup>b</sup> Subchronic and chronic inhalation NOEL = 0.5 mg/kg/day (chronic oral study of Hamada, 1987).

<sup>c</sup> Oncogenic risk was calculated as the product of the potency value,  $1.01 \times 10^{-2} \text{ mg/kg/day}^{-1}$ , and the lifetime average daily dose in mg/kg/day. As such, it is a unitless value. There was no need to incorporate a dermal absorption factor since the oncogenic potency was derived from an oral study.

**7. Ambient exposure risks**

No independent ambient exposure estimates were made by DPR (2011). The upper bound of ambient exposure risks was represented by the bystander risks, summarized above in section 6.

## 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 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 with 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 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 (short-term), subchronic (seasonal), chronic (annual) and lifetime (oncogenic) exposure to carbaryl.

#### 1. Non-oncogenic effects

##### a. Acute oral toxicity

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 of 1 mg/kg. 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 these 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

1. The rat acute gavage study of Moser (2007) which established an LED<sub>10</sub> (ED<sub>10</sub>) of 1.1 (1.5) mg/kg based on brain cholinesterase inhibition (as well as a second LED<sub>10</sub> (ED<sub>10</sub>) of 0.78 (1.11) mg/kg based on RBC cholinesterase inhibition in postnatal day 11 animals); and
2. The acute inhalation toxicity study of Weinberg (2008), which established an LED<sub>10</sub> (ED<sub>10</sub>) of 1.18 (1.70) mg/kg based on brain cholinesterase inhibition.

Both LED<sub>10</sub>s were, for all intents and purposes, equal to the critical acute NOEL of 1 mg/kg.

The major uncertainty associated with establishing the critical acute value at 1 mg/kg lay in the distinct possibility that cholinergic signs--particularly slight hypotonic gait--were present at that dose, making it a LOEL rather than a NOEL <sup>17</sup>.

However, there were also uncertainties surrounding the 0.25 mg/kg LED<sub>10</sub> determination. These included:

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 emphasized 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 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-16a 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 a critical acute 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.

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<sup>17</sup> This was explicit in the dietary assessment, where the LED<sub>10</sub> of 0.25 mg/kg provided an alternate critical value to gauge acute risk (DPR, 2010). Use of this value resulted in acute dietary MOEs that were one-fourth of those generated using 1 mg/kg. With recognition of the prominent uncertainties inherent in the lower value (see main text), DPR opted in its dietary risk assessment of carbaryl to characterize acute dietary risk using both toxicologic values.

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<sub>10</sub> value of lower than 1 mg/kg (a dose that probably 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<sub>10</sub> and ED<sub>10</sub> 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 introduced into the critical oral study and several of the support studies by utilization of gavage as the oral dosing technique. This is because food intake over a single "eating occasion" is likely to result in more gradual pesticide exposure than would occur after gavage dosing. Depending on the pharmacokinetics of carbaryl toxicity, in particular whether acute toxicity is more 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 than under gavage dosing scenarios due to the more gradual pesticide-enzyme interaction. Reactivation of cholinesterases over the exposure period would act to lessen the dietary response.

Finally, uncertainty in the acute, subchronic and chronic designations derived from a lack of measurements for more subtle neurologic and developmental effects, which may be forthcoming from effects in other neurologic systems (US EPA, 2012).

#### **b. Subchronic oral toxicity**

Because the critical subchronic oral toxicity endpoint of 0.5 mg/kg/day was established in a 1-yr chronic dog study, uncertainty existed with respect the relevance of the chronic endpoint to seasonal oral exposure scenarios. However, the alternative of estimating the subchronic oral endpoint by dividing the oral by an uncertainty factor of 10 would have resulted in an even lower value, regardless of whether 0.25 or 1 mg/kg were considered to be that endpoint.

#### **c. Chronic oral toxicity**

The critical chronic oral LED<sub>10</sub> 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 clinical signs and histopathology throughout the study, even at the high dose of 34 mg/kg/day, leaves open the possibility that the LED<sub>10</sub> value was too low, since enzyme inhibition *appeared* to be independent of overt toxicity. This uncertainty was offset by several considerations, however. First, the absence of overt toxicity did not necessarily signal an absence of toxicity. For example, subtle effects on learning or memory were not addressed. 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). And third, the possibility of chronic carbaryl-induced immunotoxicity, for which there is some evidence (*cf.*, Cranmer, 1986), was not considered for this document.

It is noted that the critical chronic oral LED<sub>10</sub> (0.5 mg/kg/day) was higher than the critical acute oral LED<sub>10</sub> (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.

**d. Acute, subchronic and chronic dermal toxicity**

There were three prominent uncertainties associated with the dermal endpoint value of 20 mg/kg/day (Austin, 2002a).

1. While this endpoint was based on brain cholinesterase inhibition, which is accepted as a legitimate risk assessment endpoint, overt toxicity was not observed even at 50 mg/kg/day. This suggests that, at least for dermal exposure, enzyme inhibition may not be an accurate indicator of toxicity.
2. On the other hand, lack of an FOB analysis left open the possibility that subtle cholinergic effects may have been missed for lack of a suitably sensitive assay. In the critical oral study, for example, slight hypotonic gait would have gone unobserved but for the execution of the FOB (Robinson and Broxup, 1997).
3. Application of a subchronic NOEL to either short-term or annual exposure scenarios may over- or underestimate the risk, respectively. It is, of course, optimal to use toxicologic studies with exposure times appropriate to the anticipated human exposure scenarios.

**e. Acute inhalation toxicity**

As with dermal and oral toxicity, acute inhalation risk was evaluated using the critical acute oral NOEL of 1 mg/kg. Outside of the uncertainties associated with designation of 1 mg/kg as the critical acute oral endpoint, additional uncertainty is attached to using an oral value to estimate inhalation risk. It is noted, however, that the inhalation LED<sub>10</sub> (ED<sub>10</sub>) of 1.18 (1.70) mg/kg, established by Weinberg (2008), was close to the acute oral NOEL of 1 mg/kg.

**f. Subchronic and chronic inhalation toxicity**

The major uncertainty in estimating risk from subchronic and chronic inhalation of carbaryl came with use of the critical chronic oral LED<sub>10</sub> of **0.5 mg/kg/day** (Hamada, 1987) as the toxicologic endpoint value. In the case of subchronic inhalation toxicity, this involved both a route extrapolation and an exposure time extrapolation. For chronic inhalation toxicity, it involved only a route extrapolation.

#### **g. 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  $<20 \times 10^6/\text{ml}$ ) 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 exposure and effect. The extent of carbaryl exposure, both with regard to time span and dose, was ill-defined and did not exclude the possibility of 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 Meeker *et al.* (2004a and 2004b) did not unambiguously attribute the presence of 1-naphthol in the urine of subfertile males to carbaryl, especially as that metabolite can also result from naphthalene exposure. The study of Wyrobek *et al.* (1981), which suggested that oligospermia was increased in carbaryl factory workers, was carried out using low subject numbers. Consequently, the measured effects showed low statistical confidence (though it might be argued that low statistical confidence in a small study could tilt the interpretation toward a positive association). A more recent examination of carbaryl factory workers from China did, however, provide corroborating 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 reproductive problems in exposed males, though the data were considered supportive rather than conclusive.

Reproductive and developmental toxicity concerns were also raised in laboratory animal studies:

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 at 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 may have a bearing particularly in the rat, where bolus dosing was most often used. Bolus exposures result in faster and higher blood levels than dietary exposure. A guideline rat dietary reproductive toxicity study did not reveal carbaryl-induced effects on F<sub>0</sub> or F<sub>1</sub> 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 did show 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 context was not clear since this species is rarely examined. Several open literature gavage studies (Rybakova, 1966; Shtenberg and Rybakova, 1968; Kitagawa *et al.*, 1977; Pant *et al.*, 1995, 1996) suggested histotoxicity in the rat male reproductive system, 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 pup health and viability through 10 days after mating of exposed males with unexposed females, though carbaryl-induced changes in two testicular enzymes, succinic dehydrogenase and adenosine triphosphatase, were noted. Osterloh *et al.* (1983) did not observe effects on testicular parameters after intraperitoneal injections of male mice out over a 5-day period. It was not clear if the negative result was due to species insensitivity, an inappropriate 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 uncertainties in the dog studies regarding both the dose responsiveness 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 relationships sufficiently convincing to set regulatory levels. For a more complete discussion of the dose responsiveness and other issues arising in the dog reproductive studies, see section IV.A.1.c. above.
2. Knaak and coworkers concluded that carbaryl metabolism in dogs differs from that in rats and humans (Knaak *et al.*, 1965, 1968; Knaak and Sullivan, 1967). Knaak considered that the dog, unlike the latter two species, does not liberate 1-naphthol for glucuronidation or sulfation. Dogs also may not 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 appeared to excrete a higher proportion 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 occurred 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 that species, 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 toxicity in dogs. Indeed, the dog system provided the critical chronic oral value, 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, especially as the presence or absence of impurities in those studies was not reported. If indeed toxicologically

significant impurities exist, their properties are not known even 40+ years after the dog studies were carried out.

Even in light of the caveats to the use of dog studies, data from rats, rabbits and mice showed developmental impacts, 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 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 those 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, reduced fetal growth and ossification delays.

#### **h. 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  $\alpha$ -naphthol (1-naphthol) may also be genotoxic, though this was also 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 underlying 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.

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*. There was, however, 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 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 consider *all* of the doses in the analysis. High dose omission might be particularly questionable in the current case, which utilized few animals, especially as a small incidence “error” in any direction at the included doses might alter the slope of the potency curve. Of even greater concern, however, was the striking possibility that inclusion of the high dose would underestimate the oncogenic potency 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 exclusion from the potency calculation was considered the most defensible course.

The assumption of low-dose linearity can be avoided by invoking a threshold and calculating an MOE. Waddell (2006) provided 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 than 100, it should be noted that oncogenic risk is rarely assessed in this way; consequently, a standard of negligible risk has not been established. USEPA’s guidance document on cancer risk assessment 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” (USEPA, 2005b), 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 relevant to the current case concerns the relationship between spontaneous incidence and chemical inducibility for particular tumors. Would a high spontaneous incidence rate translate to a high level of chemical inducibility? Or a low spontaneous rate to 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 in the National Cancer Institute SEER database was 0.21 new cases per 100,000 people (0.00021%) between 1996 and 2000; the tumors occurred 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 evident 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 is at least acknowledged.

Finally, uncertainties in the oncogenic, reproductive and developmental risk analyses arise from lack of knowledge of the effects of carbaryl degradates on these processes. This is relevant in view of the observation that residues of 1-naphthol have been detected in some food commodity samples.

## **B. 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. The MOE approach was described above in section IV.C.1. Uncertainties are introduced into MOE calculations by uncertainties in both the NOEL and exposure terms. These were documented in the preceding sections and in the accompanying exposure assessment document (DPR, 2012).

Since all of the acute inhalation MOEs in this analysis were calculated using the 1 mg/kg oral NOEL, they may underestimate inhalation risk by as much as 4-fold, since the LED<sub>10</sub> for slight hypotonic gait was determined to be 0.25 mg/kg (DPR, 2010). To provide but two examples from among the many acute inhalation exposure scenarios predicted, the inhalation MOE for aerial mixer / loaders would drop from 23 to 6, while that for aerial applicators from 182 to 46.

This consideration was also relevant to the combined (aggregate) MOE calculations, which expressed the risks associated with dermal + inhalation exposure and with dermal + inhalation + dietary exposure. As the latter route was also covered by the 1 mg/kg critical value (which was, after all, derived from an oral study), both acute inhalation and dietary risks could plausibly be evaluated using the 0.25 mg/kg LED<sub>10</sub>. In addition, the "actual" dermal NOEL may be as low as 5 mg/kg or lower, as opposed to the 20 mg/kg used in this document (see discussion of this issue above in section V.A.1.d.). Thus the effect on the combined MOE calculations of using 0.25 mg/kg for inhalation and dietary exposure and 5 mg/kg for dermal exposure could also be 4-fold. For example, high-acre liquid applicator combined MOEs would drop from 44 to 11, while high-acre granular flagger MOEs would drop from 151 to 38.

In a practical sense, choice of these plausible LEDs (inhalation and dietary) and estimated NOELs (dermal) to characterize short-term risks increases the already considerable number of scenarios in which exposure mitigation should be considered. At the very least, they emphasize the solidity of the MOEs calculated using the higher NOEL values.

### **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 under the particular exposure scenarios examined. As with non-oncogenic risk, the strength of the oncogenic risk determination rests on the confidence in both the toxicologically determined oncogenic potency value and in the lifetime exposure value, both of which have been discussed in detail in this document and in DPR (2011). Using the health protective standard of one extra case in a population of 10<sup>6</sup> individuals, carbaryl a greater than negligible risk under many exposure scenarios.

## **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.

### **1. Acute oral toxicity**

USEPA's acute "point of departure" (PoD) was 1.1 mg/kg, an LED<sub>10</sub> 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, which came from the oral study of Robinson and Broxup (1997).

### **2. Subchronic oral toxicity**

USEPA did not discuss the potential for seasonal exposure to carbaryl in their RED. DPR used the Hamada (1987) chronic dog study NOEL of 0.5 mg/kg/day to estimate subchronic oral risk.

### **3. Chronic oral toxicity**

USEPA did not estimate a chronic PoD for carbaryl because it did not consider that carbaryl, with its rapid dissociation from the cholinesterase enzyme, posed a chronic exposure risk. DPR's chronic oral LED<sub>10</sub> of 0.5 mg/kg/day (ED<sub>10</sub> = 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).

### **4. Acute, subchronic and chronic dermal toxicity**

USEPA assigned an LED<sub>10</sub> value of 30 mg/kg/day to the Austin (2002a) rat 4-wk repeat dose dermal study. DPR, using the same study, assigned a critical NOEL of 20 mg/kg/day. Both values were based on brain cholinesterase inhibition at 50 and 100 mg/kg/day.

### **5. Acute inhalation toxicity**

USEPA used their critical acute oral LED<sub>10</sub> of 1.1 mg/kg (see above) to estimate risk from acute inhalation exposure to carbaryl. DPR used the oral NOEL from the Robinson and Broxup (1997) study to estimate inhalation risk. The values are essentially equivalent.

### **6. Subchronic and chronic inhalation toxicity**

As was the case with the acute inhalation toxicity value, USEPA used the critical acute oral LED<sub>10</sub> of 1.1 mg/kg (see above) to estimate risk from subchronic and chronic inhalation exposure to carbaryl. DPR opted to use the Hamada (1987) chronic dog study NOEL of 0.5 mg/kg/day to estimate subchronic and chronic inhalation risk.

### **7. Oncogenicity**

USEPA regarded carbaryl as a "likely human carcinogen". 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 differed by a factor of 11.5-fold (USEPA:  $8.75 \times 10^{-4}$  mg/kg/day<sup>-1</sup>; DPR:  $1.01 \times 10^{-2}$  mg/kg/day<sup>-1</sup>). The major source of this discrepancy was DPR's choice to eliminate the high dose in conducting its potency analysis (see discussion in sections IV.A.2 and V.A.2).

## 8. 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 did it discuss the dog metabolism study of Knaak *et al.* (1967). A 1976 USEPA memo written by Dr. Neil Chernoff discounted the relevance of the dog data to human toxicology. The memo 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, DPR treats the dog studies at some length, and did not consider the observed maternal, fetal and perinatal toxicity to be contravened out of hand 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 studies of Smalley *et al.* (1968) and Immings *et al.* (1969) discussed above to indicate a potential developmental risk. In this regard, it is worth reiterating that carbaryl has been listed as a developmental toxin and a male reproductive toxin under Proposition 65 since August 2009.

Table V-1. Critical toxicity endpoints for carbaryl: USEPA vs. DPR

| Study type                                              | USEPA RED (USEPA, 2007a & 2007b)                                                                                                              | DPR                                                                                                                                                         |
|---------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Acute oral toxicity</b>                              | <b>Moser, 2007</b><br><b>Acute oral toxicity - rat</b><br><br>LOEL value not determined (brain ChEI)<br>LED <sub>10</sub> = 1.1 mg/kg         | <b>Robinson &amp; Broxup, 1997</b><br><b>Developmental ntx - rat</b><br><br>LOEL = 10 mg/kg (cholinergic signs)<br>NOEL = 1 mg/kg                           |
| <b>Acute, subchronic and chronic dermal toxicity</b>    | <b>Austin, 2002a</b><br><b>4-wk dermal toxicity - rat</b><br><br>LOEL = 50 mg/kg/day (brain ChEI)<br>LED <sub>10</sub> = 30 mg/kg/day         | <b>Austin, 2002a</b><br><b>4-wk dermal toxicity - rat</b><br><br>LOEL = 50 mg/kg/day (brain ChEI)<br>NOEL = 20 mg/kg/day<br>estimated NOEL = 5 mg/kg/day    |
| <b>Acute inhalation toxicity</b>                        | <b>Moser, 2007</b><br><b>Acute oral toxicity - rat</b><br><br>LOEL value not determined (brain ChEI)<br>LED <sub>10</sub> = 1.1 mg/kg         | <b>Robinson &amp; Broxup, 1997</b><br><b>Developmental ntx - rat</b><br><br>LOEL = 10 mg/kg (cholinergic signs)<br>NOEL = 1 mg/kg                           |
| <b>Subchronic and chronic inhalation toxicity</b>       | <b>Moser, 2007</b><br><b>Acute oral toxicity - rat</b><br><br>LOEL value not determined (brain ChEI)<br>LED <sub>10</sub> = 1.1 mg/kg         | <b>Hamada, 1987</b><br><b>1-year chronic - dog</b><br><br>LOEL = 3.1 mg/kg/day (brain / RBC / plasma ChEI)<br>LED <sub>10</sub> = 0.5 mg/kg/day             |
| <b>Subchronic oral toxicity</b>                         | n/a (USEPA does not discuss intermediate / seasonal oral exposure scenarios for carbaryl)                                                     | <b>Hamada, 1987</b><br><b>1-year chronic - dog</b><br><br>LOEL = 3.1 mg/kg/day (brain / RBC / plasma ChEI)<br>LED <sub>10</sub> = 0.5 mg/kg/day             |
| <b>Chronic toxicity (oral, dermal &amp; inhalation)</b> | n/a (USEPA does not consider carbaryl to pose a chronic toxicity risk)                                                                        | <b>Hamada, 1987</b><br><b>1-year chronic - dog</b><br><br>LOEL = 3.1 mg/kg/day (brain / RBC / plasma ChEI)<br>LED <sub>10</sub> = 0.5 mg/kg/day             |
| <b>Oncogenicity</b>                                     | <b>Hamada, 1993b</b><br><b>2-year chronic / onco - mouse</b><br><br>Dose-dependent hemangiosarcomas<br>95% UB potency = $8.75 \times 10^{-4}$ | <b>Hamada, 1993b</b><br><b>2-year chronic / onco - mouse</b><br><br>Dose-dependent hemangiosarcomas & hemangiomas<br>95% UB potency = $1.01 \times 10^{-2}$ |

## VI. ISSUES RELATED TO THE FOOD QUALITY PROTECTION ACT

The Food Quality Protection Act (FQPA) 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. Based on the analysis provided in DPR’s dietary assessment of carbaryl (DPR, 2010), which showed MOEs of less than 100 for three infant or age 1-2 yr subpopulations, it appears that the extra 10-fold factor should be considered.

FQPA also requires the USEPA to 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.

### A. AGGREGATE EXPOSURE AND RISK

DPR’s exposure assessment document indicates a potential for exposure to carbaryl by the oral, dermal and inhalation routes (DPR, 2012). Since toxicologic impacts are plausible by all of these routes, an aggregate health assessment was undertaken to determine the risks associated with simultaneous exposure by more than one route.

Aggregate analysis revealed only one non-occupational scenario, short-term exposures to adult bystanders, in which contributing MOEs (inhalation = 110; dietary = 228) resulted in a combined MOE of 74 (Table xxxl). It is worth noting, however, that MOEs also dipped below 100 for four handler scenarios in which the individual contributing MOEs were greater than 100: (1) short-term groundboom applicators (dermal + inhalation + dietary); (2) short-term high-acre broadcast spreader applicators (dermal + inhalation + dietary); (3) seasonal airblast citrus applicators (dermal + inhalation); and (4) annual high-pressure handwand mixer / loader / applicators (dermal + inhalation). These are indicated by the underlined values in the “Aggregate” MOE column in Table IV-7a. Many other combined exposure MOEs were also below 100, but in each of the latter cases at least one of the individual contributing MOEs was already below 100.

Aggregate calculations relating to non-occupational oncogenic risk were considered unnecessary since all reported values already exceeded the health protective benchmark of one extra cancer in  $10^6$  exposed individuals. Aggregate MOEs that also included carbaryl in ambient air were not included in this analysis due to the lack of ambient air measurements. It is assumed that, were such measurements available, they would add slightly to the aggregate risks (*i.e.*, lower the aggregate MOEs).

## B. CUMULATIVE EXPOSURE AND RISK

US EPA completed its "Revised N-Methyl Carbamate Cumulative Risk Assessment" (CRA) in 2007 (US EPA, 2007c). The following carbamates were included in the US EPA CRA, based on "their shared ability to inhibit acetylcholinesterase (AChE) by carbamylation of the serine hydroxyl group located in the active site of the enzyme" (p. 2): carbaryl, aldicarb, oxamyl, formetanate-HCl, methomyl, carbofuran, propoxur, methiocarb, thiodicarb and pirimicarb. Three potential exposure pathways were identified: food, drinking water and residential / non-occupational (occupational exposure was not included). The CRA was executed for acute, single day exposures using the following steps (quoted directly from the US EPA document, pages 3-4):

- Selection of an index chemical to use as the point of reference to standardize the toxic potencies of each NMC, determination of the relative toxic contribution of each NMC, and establishment of a value to estimate potential risk for the group (*i.e.*, point of departure).
- Evaluation of interspecies differences (*i.e.*, extrapolation of rat responses to human responses); intraspecies variability; and the potential sensitivity to infants and children.
- Estimation of the risks associated with all pertinent pathways of exposure (*i.e.*, food, drinking water, residential) in a manner that is both realistic and reflective of variability due to differences in location, time, and demographic characteristics of exposed groups.
- Identification of the significant contributors to risk.
- Characterization of the confidence in the results and the uncertainties associated with the assessment.

The relative potency factor approach was used to determine cumulative risk. Oxamyl was selected as the index chemical in light of "its high quality dose response data for all routes of exposure, as well as high quality time-to-recovery data" (p. 4). The toxicologic endpoint was the peak level of brain AChE inhibition following gavage exposure in rats. Inhibition data were modeled using the benchmark dose approach, with the benchmark response set at 10%. US EPA stated that 10% inhibition was not associated with functional or behavioral neurotoxicity. Interspecies and FQPA safety factors were applied mathematically to the relative potency factor for each chemical when warranted by lack of specific data. The standard intraspecies factor of 10 was applied to all of the compounds, making 10 the target MOE for the overall CRA. Exposure profiles from food, drinking water and residential and other non-occupational settings were developed for each chemical, taking into account the possibilities of overlap, co-occurrence or variance between chemicals and identifying populations at potential risk of exposure.

Multipathway MOEs for children 1-2 years and 3-5 years at the 99.9th exposure percentile were 8 and 9, respectively. As the dominant exposure pathway, food was the major contributor to risk. US EPA concluded in light of recent risk mitigation efforts and key risk assessment assumptions that minimized the potential to underestimate risk, that there is a "reasonable certainty that no harm" will result from cumulative exposure to the NMC pesticides covered by its assessment.

### **C. 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 and 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<sub>2</sub> 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 use. 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.

### **D. 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) AND REFERENCE CONCENTRATIONS (RfC)

### A. REFERENCE DOSES (RfD) - ORAL EXPOSURE

Oral doses of carbaryl below a calculated reference dose ( $RfD_{oral}$ ) were considered unlikely to pose a risk to human health. RfDs were calculated for acute, subchronic and chronic dietary exposure scenarios by dividing the critical oral NOELs by an uncertainty factor of 100, which was a product of the 10x interspecies and 10x intraspecies uncertainty factors. All of the uncertainties that accompanied selection of this endpoint were applicable to this calculation (see section V.A.). The oral RfD calculated below was most relevant to the general population exposed through the diet. Two such RfDs ( $RfD_{acute\#1}$  and  $RfD_{acute\#2}$ ) 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, respectively. These values along with other RfDs and RfCs appear in Table VII-1.

$$RfD_{oral} = \text{critical oral NOEL} \div 100$$

❖ To calculate the acute oral reference dose #1 ( $RfD_{acute\#1}$ ):

Critical acute oral NOEL = 1 mg/kg:

$$RfD_{acute\#1} = 1 \text{ mg/kg} \div 100 = \mathbf{0.01 \text{ mg/kg}}$$

❖ To calculate the acute oral reference dose #2 ( $RfD_{acute\#2}$ ):

Critical acute oral  $LED_{10}$  = 0.25 mg/kg:

$$RfD_{acute\#2} = 0.25 \text{ mg/kg} \div 100 = \mathbf{0.0025 \text{ mg/kg}}$$

❖ To calculate the seasonal / annual oral reference dose ( $RfD_{s/a}$ ):

Critical subchronic / chronic oral NOEL = 0.5 mg/kg/day:

$$RfD_{s/a} = 0.5 \text{ mg/kg/day} \div 100 = \mathbf{0.005 \text{ mg/kg/day}}$$

### B. REFERENCE DOSES (RfD) - DERMAL EXPOSURE

As with the oral RfDs, dermal doses of carbaryl below a calculated reference dose ( $RfD_{dermal}$ ) were considered unlikely to pose a risk to human health. The  $RfD_{dermal}$  was calculated for acute and subchronic exposure scenarios by dividing the estimated critical dermal NOEL, 20 mg/kg/day (Austin, 2002a), by an uncertainty factor of 100. An extra uncertainty factor of 10 should be considered to account for the lack of a chronic dermal study.

$$RfD_{dermal} = (\text{critical dermal NOEL} \div 100) \times 5/7$$

❖ To calculate the acute /seasonal / chronic dermal reference dose ( $RfD_{a/s}$ ):

Critical acute / subchronic NOEL = 20 mg/kg/day

$$RfD_{a/s} = (20 \text{ mg/kg/day} \div 100) \times 5/7 = \mathbf{0.14 \text{ mg/kg/day}}$$

## C. REFERENCE CONCENTRATIONS (RfC) - INHALATION EXPOSURE

### 1. Acute inhalation reference concentrations

Acute RfCs for infants and adults were calculated from the rat acute oral LED<sub>10</sub> of 1 mg/kg. The possibility that 0.25 mg/kg might be a more appropriate critical endpoint value is acknowledged. This was accomplished by converting the LED<sub>10</sub> to an air concentration using the default human breathing rate appropriate to the exposure time (1, 8 or 24 hours)<sup>18</sup>, then dividing by a combined uncertainty factor of 100 (10x interspecies and 10x intraspecies uncertainty factors).

❖ For infants (considered to represent all children), RfCs for 1, 8 and 24 hr exposure times were calculated as follows:

$$\text{RfC}_{1\text{-hr}} = (1 \text{ mg/kg} \div 0.25 \text{ m}^3/\text{kg/hr}) \div 100 = \mathbf{0.04 \text{ mg/m}^3}$$

$$\text{RfC}_{8\text{-hr}} = (1 \text{ mg/kg} \div 0.20 \text{ m}^3/\text{kg}/8\text{-hr}) \div 100 = \mathbf{0.05 \text{ mg/m}^3}$$

$$\text{RfC}_{24\text{-hr}} = (1 \text{ mg/kg} \div 0.59 \text{ m}^3/\text{kg}/24\text{-hr}) \div 100 = \mathbf{0.02 \text{ mg/m}^3}$$

❖ For adults, RfCs for 1, 8 and 24 hr exposure times were calculated as follows:

$$\text{RfC}_{1\text{-hr}} = (1 \text{ mg/kg} \div 0.045 \text{ m}^3/\text{kg/hr}) \div 100 = \mathbf{0.22 \text{ mg/m}^3}$$

$$\text{RfC}_{8\text{-hr}} = (1 \text{ mg/kg} \div 0.093 \text{ m}^3/\text{kg}/8\text{-hr}) \div 100 = \mathbf{0.11 \text{ mg/m}^3}$$

$$\text{RfC}_{24\text{-hr}} = (1 \text{ mg/kg} \div 0.28 \text{ m}^3/\text{kg}/24\text{-hr}) \div 100 = \mathbf{0.04 \text{ mg/m}^3}$$

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<sup>18</sup> The following default breathing rates were assumed (Andrews and Patterson, 2000): resting human infant (applicable to 8- and 24-hr exposure times): 0.59 m<sup>3</sup>/kg/day; active human infant (1-hr exposure time): 0.25 m<sup>3</sup>/mg/hr; resting human adult (8- and 24-hr exposure times): 0.28 m<sup>3</sup>/kg/day; active human adult (1-hr exposure time): 0.045 m<sup>3</sup>/mg/hr.

## 2. Seasonal and annual inhalation reference concentrations ( $RfC_{s/a}$ )

For seasonal-annual  $RfCs$  ( $RfC_{s/a}$ ), which were based on the critical rat subchronic oral NOEL of 0.5 mg/kg/day, a human equivalent value first was calculated for infants and adults using their respective default breathing rates, then the intra- and interspecies uncertainty factor of 100 was applied.

$$RfC_{s/a} = (\text{critical subchronic-chronic oral NOEL} \div \text{human default inhalation rate}) \div 100$$

❖ For infants (considered to represent all children):

$$RfC_{s/a} = (0.5 \text{ mg/kg/day} \div 0.59 \text{ m}^3/\text{kg/day}) \div 100 = \mathbf{0.008 \text{ mg/m}^3/\text{day}}$$

❖ For adults:

$$RfC_{s/a} = (0.5 \text{ mg/kg/day} \div 0.28 \text{ m}^3/\text{kg/day}) \div 100 = \mathbf{0.02 \text{ mg/m}^3/\text{day}}$$

Table VII-1. Oral and dermal reference doses (RfDs), inhalation reference concentrations (RfCs) and anticipated exposures to carbaryl

| Exposure time and species                                                                        | Endpoint                                                      | LOEL and NOEL (or LED)                                                                  | RfD or RfC                                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------|-----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Acute oral #1</b><br>Rat gavage dvpmt. ntx. study, gd 6 - ppd 10 (Robinson & Broxup, 1997)    | cholinergic signs, brain ChEI and body weight gain decrements | <b>LOEL</b><br>10 mg/kg<br><br><b>NOEL</b><br>1 mg/kg                                   | <b>RfD<sub>acute#1</sub></b><br>0.01 mg/kg                                                                                                                                                                                                                                                                                                                                        |
| <b>Acute oral #2</b><br>Rat gavage dvpmt. ntx. study, gd 6 - ppd 10 (Robinson & Broxup, 1997)    | slight hypotonic gait                                         | <b>LOEL</b><br>not determined <sup>a</sup><br><br><b>LED<sub>10</sub></b><br>0.25 mg/kg | <b>RfD<sub>acute#2</sub></b><br>0.0025 mg/kg                                                                                                                                                                                                                                                                                                                                      |
| <b>Seasonal-annual oral</b><br>Dog 1-yr dietary study (Hamada, 1987)                             | brain ChEI                                                    | <b>LOEL</b><br>3.4 mg/kg/day<br><br><b>LED<sub>10</sub></b><br>0.5 mg/kg/day            | <b>RfD<sub>s/a</sub></b><br>0.005 mg/kg/day                                                                                                                                                                                                                                                                                                                                       |
| <b>Dermal</b><br>Rabbit 4-wk dermal study (Austin, 2002a)                                        | brain ChEI                                                    | <b>LOEL</b><br>50 mg/kg<br><br><b>NOEL</b><br>20 mg/kg <sup>c</sup>                     | <b>RfD<sub>a/s</sub></b><br>0.14 mg/kg                                                                                                                                                                                                                                                                                                                                            |
| <b>Acute inhalation</b><br>Rat gavage dvpmt. ntx. study, gd 6 - ppd 10 (Robinson & Broxup, 1997) | cholinergic signs, brain ChEI and body weight gain decrements | <b>LOEL</b><br>10 mg/kg<br><br><b>NOEL</b><br>1 mg/kg                                   | <b>Infants:</b><br><b>RfC<sub>1-hr</sub></b><br>0.04 mg/m <sup>3</sup><br><b>RfC<sub>8-hr</sub></b><br>0.05 mg/m <sup>3</sup><br><b>RfC<sub>24-hr</sub></b><br>0.02 mg/m <sup>3</sup><br><br><b>Adults:</b><br><b>RfC<sub>1-hr</sub></b><br>0.22 mg/m <sup>3</sup><br><b>RfC<sub>8-hr</sub></b><br>0.11 mg/m <sup>3</sup><br><b>RfC<sub>24-hr</sub></b><br>0.04 mg/m <sup>3</sup> |
| <b>Seasonal-annual inhalation</b><br>Dog 1-yr dietary study (Hamada, 1987)                       | brain ChEI                                                    | <b>LOEL</b><br>3.4 mg/kg/day<br><br><b>LED<sub>10</sub></b><br>0.5 mg/kg/day            | <b>RfD<sub>s/a</sub> - infants</b><br>0.008 mg/m <sup>3</sup><br><br><b>RfD<sub>s/a</sub> - adults</b><br>0.02 mg/m <sup>3</sup>                                                                                                                                                                                                                                                  |

<sup>a</sup> A clear increase in incidence of slight hypotonic gait (and other signs) was observed at 10 mg/kg; an increase was also observed at 1 mg/kg, though it was less certain. As a consequence, the incidence data were subjected to benchmark dose analysis, generating an alternative acute regulatory value.

<sup>b</sup> PE, point estimate; MC, Monte Carlo estimate. The dietary subpopulations examined are those covered in the DEEM-FCID<sup>®</sup> 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.

<sup>c</sup> The critical NOEL for dermal toxicity was estimated based on brain cholinesterase inhibition in the 21-day dermal toxicity study of Austin (2002a). An extra 10-fold uncertainty factor should be considered for annual exposure.

## IX. CONCLUSIONS

Health risks to humans from exposure to carbaryl were assessed by combining toxicity studies conducted in laboratory animals with exposure projections for humans under occupational and bystander conditions. Since short-term, seasonal, annual and lifetime exposures were expected, corresponding risk values for each of these scenarios were calculated.

In general, margins of exposure (MOEs) of 100 or greater were considered sufficient to protect human health against non-oncogenic effects when the critical NOELs were based on studies in laboratory animals, as was the case in this document. In addition, an increase in the cancer incidence rate of less than one per  $10^6$  exposed individuals (i.e., an incidence rate of  $10^{-6}$ ) was considered a negligible risk.

MOEs of less than 100 were calculated for dermal, inhalation and aggregate (including dietary) exposures under short-term, seasonal and annual exposure scenarios. This was particularly true of occupational handler and reentry workers under short-term and seasonal exposure conditions, where MOEs of less than 1 were observed in some instances and MOEs less than 100 in many instances. Residential reentry onto carbaryl-treated turf also generated very low MOEs ( $\leq 5$ ) for both children and adults (dermal, short-term), as did short-term exposures for some residential handlers and short-term bystanders to agricultural and public pest control applications. Oncogenic risk exceeded the negligible risk standard of  $10^{-6}$  by orders of magnitude among occupational handler and reentry workers, approaching or equalling  $10^{-2}$  in some cases. Oncogenic risk for adult bystanders of agricultural applications also exceeded  $10^{-6}$  (i.e.,  $1.88 \times 10^{-6}$ ). Risk mitigation measures should be considered.

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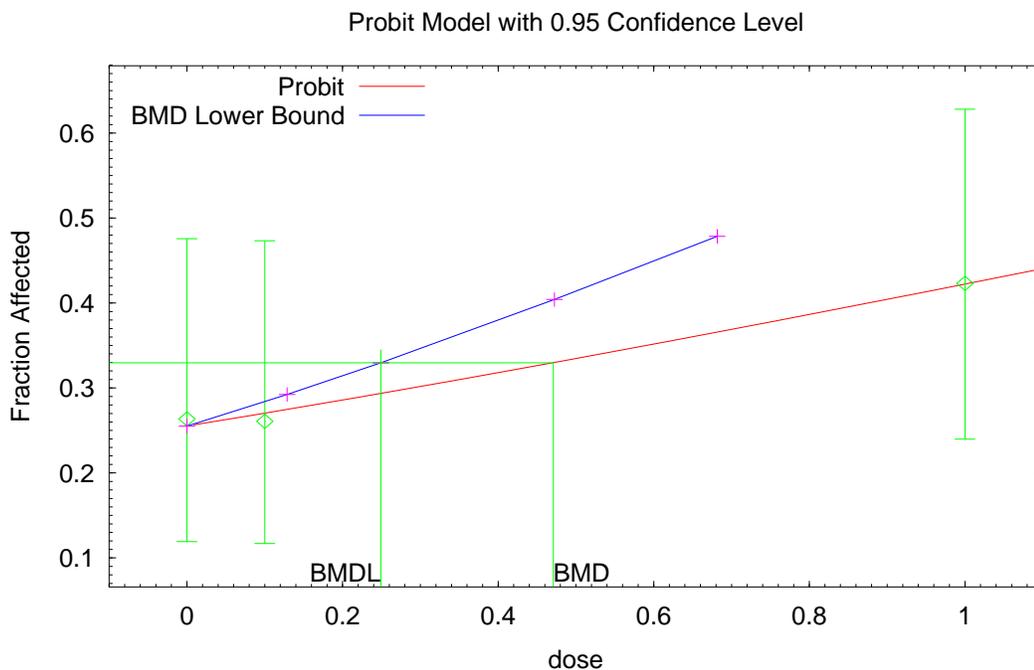
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**Appendix I. Benchmark dose extrapolation for induction of slight hypotonic gait in pregnant Sprague-Dawley rats (Robinson and Broxup, 1997)**



09:39 05/06 2009

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:

$$P[\text{response}] = \text{CumNorm}(\text{Intercept} + \text{Slope} * \text{Dose}),$$

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<br>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

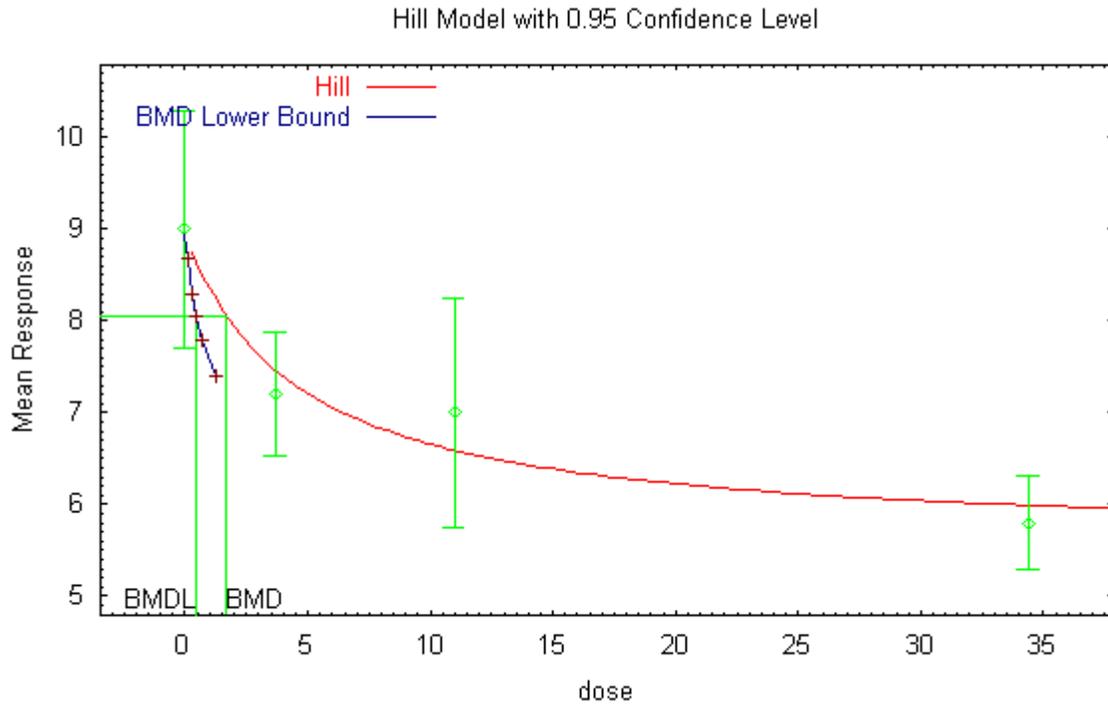
Risk Type    =    Extra risk

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)**



15:53 12/05 2005

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

**BMDS MODEL RUN**

The form of the response function is:

$$Y[\text{dose}] = \text{intercept} + v \cdot \text{dose}^n / (k^n + \text{dose}^n)$$

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         | 1 | 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:  $Y_{ij} = \mu(i) + e(ij)$   
 $\text{Var}\{e(ij)\} = \sigma^2$

Model A2:  $Y_{ij} = \mu(i) + e(ij)$   
 $\text{Var}\{e(ij)\} = \sigma(i)^2$

Model R:  $Y_i = \mu + e(i)$   
 $\text{Var}\{e(i)\} = \sigma^2$

Degrees of freedom for Test A1 vs fitted  $\leq 0$

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

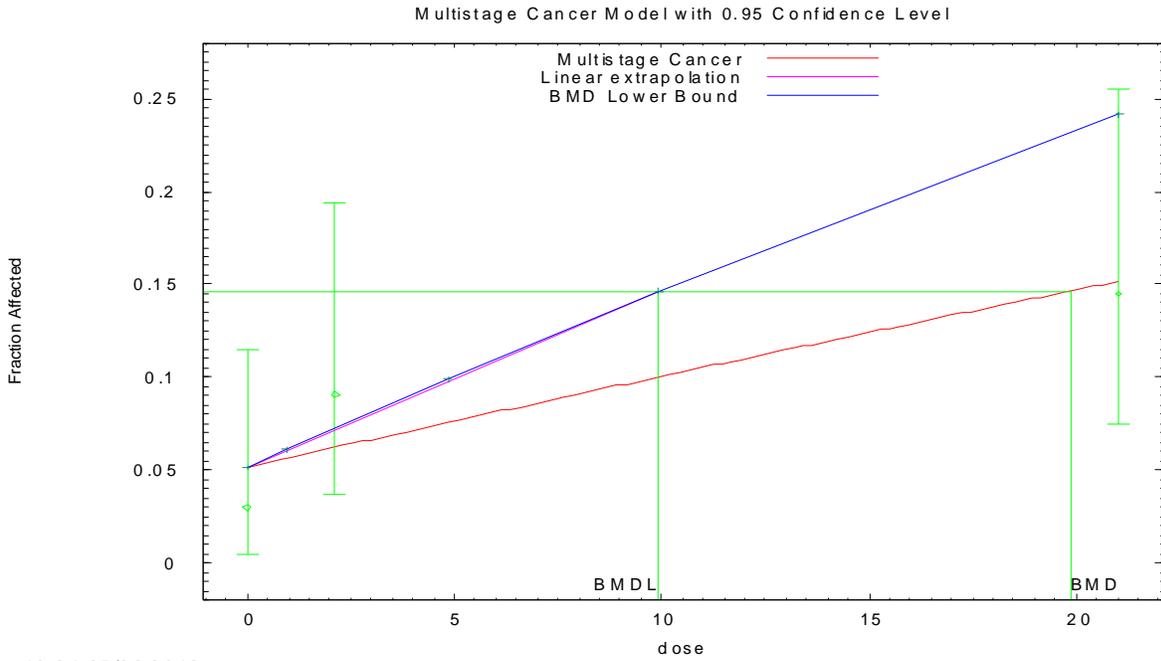
Confidence level = 0.95

BMD = 1.69014

BMDL = 0.46739

**Appendix III. Benchmark dose extrapolation for hemangiosarcoma / hemangioma data in male mice (Hamada, 1993b)**

Multistage cancer model; top dose deleted  
 Multistage Cancer Slope Factor = 0.0100587



10:34 05/02 2012

Hamada (1993b) mouse 2-yr dietary oncogenicity study with carbaryl  
 Hemangiosarcoma / hemangioma data in males  
 Multistage cancer model  
 Risk type: "Extra risk"

=====  
 Multistage Cancer Model. (Version: 1.9; Date: 05/26/2010)  
 Input Data File: C:/USEPA/BMDS212/Data/msc\_Hamada (1993b) mouse tumors carbaryl,  
 human, no top dose\_Opt.(d)  
 Gnuplot Plotting File: C:/USEPA/BMDS212/Data/msc\_Hamada (1993b) mouse tumors  
 carbaryl, human, no top dose\_Opt.plt  
 Wed May 02 10:34:54 2012  
 =====

BMDS\_Model\_Run

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose}^{\beta_2} - \beta_2 * \text{dose}^2)]$$

The parameter betas are restricted to be positive

Dependent variable = Col3  
Independent variable = Col1

Total number of observations = 3  
Total number of records with missing values = 0  
Total number of parameters in model = 3  
Total number of specified parameters = 0  
Degree of polynomial = 2

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

Background = 0.0548458  
Beta(1) = 0.00490187  
Beta(2) = 0

Asymptotic Correlation Matrix of Parameter Estimates

( \*\*\* The model parameter(s) -Beta(2)  
have been estimated at a boundary point, or have been specified by the user,  
and do not appear in the correlation matrix )

|            | Background | Beta(1) |
|------------|------------|---------|
| Background | 1          | -0.64   |
| Beta(1)    | -0.64      | 1       |

Parameter Estimates

| Variable   | Estimate   | Std. Err. | 95.0% Wald Confidence Interval |                   |
|------------|------------|-----------|--------------------------------|-------------------|
|            |            |           | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0.0516724  | *         | *                              | *                 |
| Beta(1)    | 0.00530042 | *         | *                              | *                 |
| Beta(2)    | 0          | *         | *                              | *                 |

\* - Indicates that this value is not calculated.

Analysis of Deviance Table

| Model         | Log(likelihood) | # Param's | Deviance | Test d.f. | P-value |
|---------------|-----------------|-----------|----------|-----------|---------|
| Full model    | -57.6212        | 3         |          |           |         |
| Fitted model  | -58.4016        | 2         | 1.56078  | 1         | 0.2116  |
| Reduced model | -60.6016        | 1         | 5.96091  | 2         | 0.05077 |

AIC: 120.803

Goodness of Fit

| Dose    | Est._Prob. | Expected | Scaled   |      | Residual |
|---------|------------|----------|----------|------|----------|
|         |            |          | Observed | Size |          |
| 0.0000  | 0.0517     | 3.410    | 2.000    | 66   | -0.784   |
| 2.1200  | 0.0623     | 4.110    | 6.000    | 66   | 0.963    |
| 21.0200 | 0.1517     | 10.464   | 10.000   | 69   | -0.156   |

Chi<sup>2</sup> = 1.57    d.f. = 1    P-value = 0.2107

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 19.8778

BMDL = 9.94168

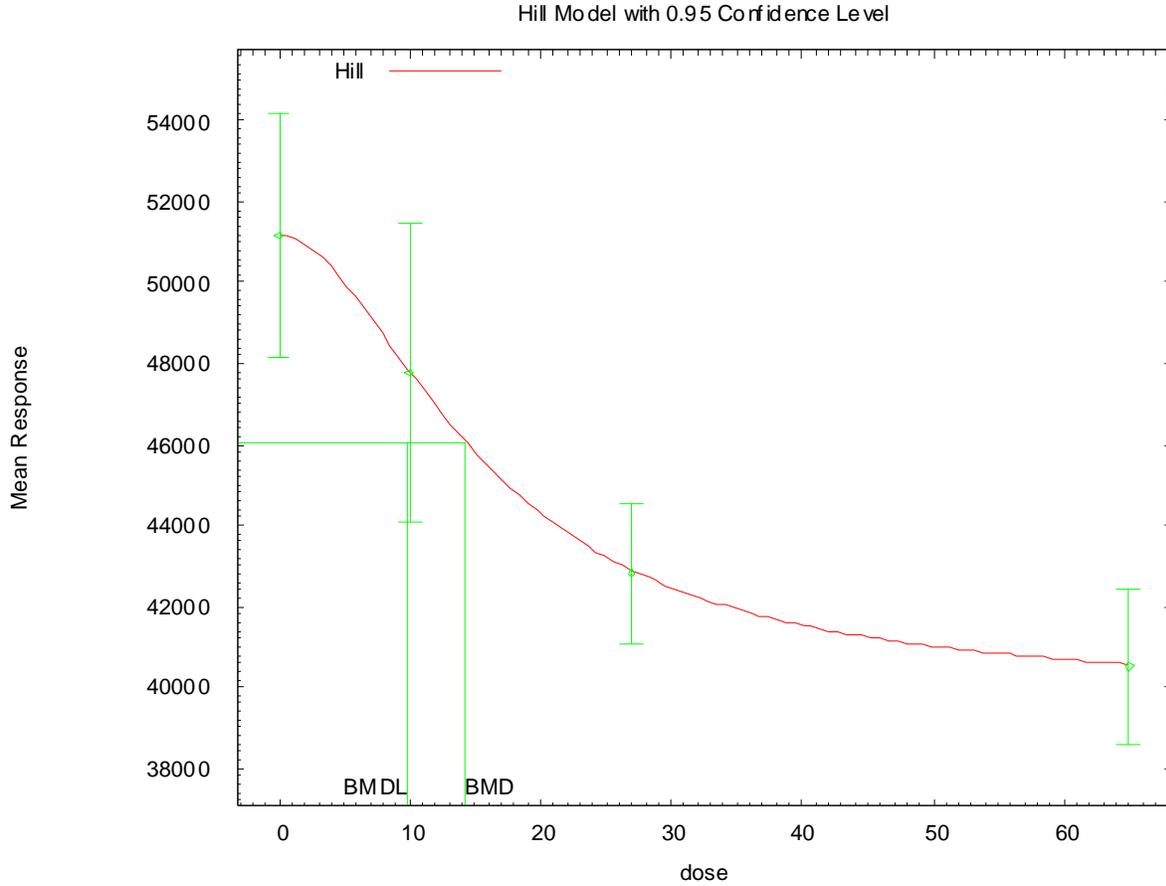
BMDU = 98.985

Taken together, (9.94168, 98.985 ) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0100587

Warning: BMDL is out of the three times range of dose for some BMR in BMDL curve computation.

**Appendix IV. Benchmark dose analysis for brain cholinesterase inhibition data in female rats following acute inhalation exposure (Weinberg, 2008)**



Weinberg (2008) rat acute inhalation study with carbaryl  
 Brain cholinesterase inhibition in females  
 Hill model  
 Risk type: "Relative" risk

```

=====
Hill Model. (Version: 2.15; Date: 10/28/2009)
Input Data File: C:/USEPA/BMDS212/Data/hil_Weinberg (2008) female bChE_Opt.(d)
Gnuplot Plotting File: C:/USEPA/BMDS212/Data/hil_Weinberg (2008) female bChE_Opt.plt
Tue May 24 16:53:41 2011
=====
  
```

**BMDS Model Run**

~~~~~

The form of the response function is:

$$Y[\text{dose}] = \text{intercept} + v \cdot \text{dose}^n / (k^n + \text{dose}^n)$$

Dependent variable = Col3  
 Independent variable = Col1  
 rho is set to 0  
 Power parameter 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  
 intercept = 51181  
 v = -10675  
 n = 2.06485  
 k = 16.6463

Asymptotic Correlation Matrix of Parameter Estimates

( \*\*\* The model parameter(s) -rho  
 have been estimated at a boundary point, or have been specified by the user,  
 and do not appear in the correlation matrix )

	alpha	intercept	v	n	k
alpha	1	0.00012	-0.00026	-0.00021	0.00015
intercept	0.00012	1	-0.49	-0.22	-0.29
v	-0.00026	-0.49	1	0.8	-0.58
n	-0.00021	-0.22	0.8	1	-0.55
k	0.00015	-0.29	-0.58	-0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	3.78581e+006	1.19718e+006	1.43938e+006	6.13224e+006

intercept	51181.3	870.147	49475.9	52886.8
v	-11447.5	2075.19	-15514.8	-7380.19
n	1.86266	0.804849	0.285189	3.44014
k	15.8617	4.54105	6.96143	24.762

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	5.12e+004	5.12e+004	2.41e+003	1.95e+003	-0.000395
10	5	4.78e+004	4.78e+004	2.97e+003	1.95e+003	0.000158
27	5	4.28e+004	4.28e+004	1.4e+003	1.95e+003	-0.000356
65	5	4.05e+004	4.05e+004	1.53e+003	1.95e+003	0.000593

Degrees of freedom for Test A3 vs fitted <= 0

Model Descriptions for likelihoods calculated

Model A1:  $Y_{ij} = \mu(i) + e(ij)$   
 $\text{Var}\{e(ij)\} = \sigma^2$

Model A2:  $Y_{ij} = \mu(i) + e(ij)$   
 $\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3:  $Y_{ij} = \mu(i) + e(ij)$   
 $\text{Var}\{e(ij)\} = \sigma^2$   
 Model A3 uses any fixed variance parameters that were specified by the user

Model R:  $Y_i = \mu + e(i)$   
 $\text{Var}\{e(i)\} = \sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-161.467702	5	332.935403
A2	-159.577513	8	335.155026
A3	-161.467702	5	332.935403
fitted	-161.467702	5	332.935404
R	-178.674837	2	361.349673

### Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?  
(A2 vs. R)

Test 2: Are Variances Homogeneous? (A1 vs A2)

Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

(Note: When  $\rho=0$  the results of Test 3 and Test 2 will be the same.)

### Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	38.1946	6	<.0001
Test 2	3.78038	3	0.2862
Test 3	3.78038	3	0.2862
Test 4	6.5897e-007	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 .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

NA - Degrees of freedom for Test 4 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 = 14.1523

BMDL = 9.81235

