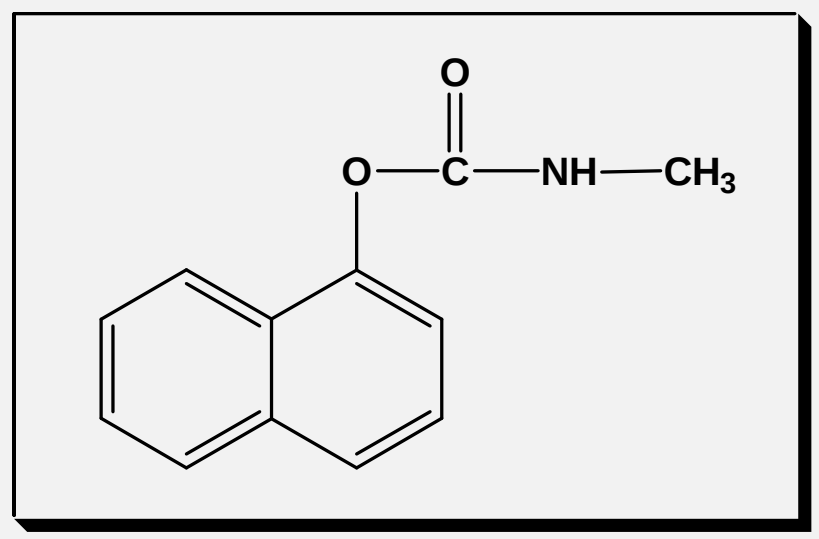
Carbaryl
(1-naphthyl methylcarbamate)

OCCUPATIONAL AND BYSTANDER
RISK CHARACTERIZATION DOCUMENT



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ABBREVIATIONS

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

VAP	mathematically smoothed velocity
VCL	curvilinear velocity
VSL	straight line velocity
WH&S	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 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 in the United States in 1959 for use on cotton. 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_2 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_2 (detectable when carbaryl is labeled in the carbonyl or N-methyl carbon, but not when labeled in the naphthalene ring). Metabolites were conjugated with sulfate or glucuronic acid. For animals receiving 1 mg/kg, about half 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-^{14}C]-carbaryl in the blood and other tissues after exposure by the oral (1.08 or 8.45 mg/kg), dermal (17.25 or 102.95 mg/kg) and intravenous (0.80 or 9.20 mg/kg) routes. Peak levels of radioactivity were detected in the blood at 15 and 30 min for the low and high dose oral treatments, respectively; at 4 and 12 hr for the dermal applications; and were already maximal by the first time point (5 min) for the iv injections. *Oral dosing*: by 24 hr, radioactivity levels had decreased to 0.81%-2.4% of peak levels in blood fractions (both doses), 0.60%-2.4% in brain (both doses), 0.67% in liver (high dose only) and 0.32% in fat (high dose only). *Dermal dosing*: by 24 hr, radioactivity levels had decreased to 15.9%-25.8% of peak levels in blood fractions (both doses), 27.1%-30.6% in brain (both doses), 24.4% in liver (high dose only) and 15.6% in fat (high dose only). *IV dosing*: by 24 hr, radioactivity levels had decreased to 4.6%-10.5% in blood fractions (both doses), 1.1%-1.3% in brain (both doses), 5.7% in liver (high dose only) and 0.72% in fat (high dose only).

The recovery kinetics in other laboratory species (guinea pig, sheep) were generally similar to those in the rat. However, there were 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_{50}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 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 of 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 (albeit with weaker experimental support) resulted in an alternative critical acute LED_{10} 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_{10} of 1.1 mg/kg based on inhibition of brain cholinesterase activity in postnatal day 11 rats following a single gavage exposure to carbaryl.

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 and 20% in females at 3.7 mg/kg/day ($p<0.05$). RBC cholinesterase showed statistically significant inhibition at the mid and high doses (11.0-♂ / 11.2-♀ mg/kg/day and 33.8-♂ / 34.4-♀ mg/kg/day, respectively) at all treatment intervals (weeks 5, 13, 26 and 52), while non-statistically significant inhibition was detected at the low dose (up to 14% in males at week 13 and 13% in females at week 5, the first measurement). Plasma cholinesterase 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_{10} 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_{10} for brain cholinesterase inhibition in females using the Hill algorithm was **0.5 mg/kg/day** (ED_{10} = 1.7 mg/kg/day). This value was used to evaluate the non-oncogenic risks from annual (*i.e.*, chronic) exposure to carbaryl.

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_{10} (ED_{10}) of 9.81 (14.15) mg/m^3, 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^3. However, the oral NOEL was considered primary because it was based not only on enzyme inhibition, but on overt cholinergic signs as well (see above).

Subchronic and chronic inhalation toxicity. As neither a subchronic nor a chronic inhalation study was available, the critical chronic oral LED_{10} of **0.5 mg/kg/day** from the 1-yr dog dietary study was chosen to evaluate these inhalation exposure scenarios.

Genotoxicity. Carbaryl tested positive in one of five gene mutation studies, four of six chromosomal aberration studies and two of four DNA damage studies reviewed for this assessment. It should thus be viewed as potentially genotoxic. Virtually all of the positive studies were performed *in vitro*, which made them less relevant than *in vivo* studies to the whole organism. One study in V79 Chinese hamster fibroblasts showed that, like carbaryl, the carbaryl metabolite ∝-naphthol (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 value (or Multistage Cancer Slope value) was **9.72×10^{-3} mg/kg/day^{-1}**.

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 $<20 \times 10^6$/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. With the exception of possible developmental delays that were 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 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. An additional uncertainty factor related to possible developmental or reproductive effects was not considered for this document, though such sensitivities may exist.

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. For short-term inhalation

and oral exposures, the critical NOEL of 1 mg/kg was used, with the understanding that the alternative LED_{10} of 0.25 mg/kg would result in MOEs that were reduced by a factor of 4.

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 4.05×10^{-3} for airblast mixer / loaders (handlers) and 1.38×10^{-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 duster 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: 1.81×10^{-6}

Ambient risk: No independent ambient exposure estimates were estimated. The upper bound of ambient exposure risks was represented by the bystander risks as summarized above.

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. Aggregate oncogenicity by the dermal, inhalation and dietary routes was also measured in handlers and reentry workers. These additive values accentuated the already very high oncogenic risk posed by carbaryl.

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_{50} 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 those 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 studies gave negative results. However, 1-naphthol, like carbaryl, induced an aberrant form of mitosis called c-mitosis in V79 cells that may reflect effects on mitotic spindle formation.

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

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 acaracide, though registrations as a fungicide, herbicide, miticide, disinfectant and repellent are currently inactive. 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. Some 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 in the United States in 1959 for use on cotton (USEPA, 2004). More than 100 food tolerances are in effect for this chemical. USEPA has designated carbaryl to be a General Use Pesticide. After conducting a Special Review of carbaryl, USEPA concluded in 1980 that toxicologic concerns, particularly those relating to teratogenicity, did not warrant cancellation. A Registration Standard promulgated in 1984 and revised in 1988 specified the requirements for continued registration (USEPA, 2004).

USEPA's Interim Reregistration Eligibility Decision (IRED), issued in October 2004, cited possible health risks associated with residential and occupational exposures. This triggered mitigation directives relating to personal protective gear, engineering controls, cancellation of residential aerosol products, packaging and application requirements for residential use and restriction of residential lawn applications to spot treatment (USEPA, 2004, 2007a). The IRED also cited possible impacts on non-target organisms and endangered species that may require mitigation. 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 IRED. 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 (initially reported as fulfilling the requirements for carcinogen listing in 2009 [OEHHA, 2009a]). 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_{10} 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_{10} 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^{-3} mg/kg/day^{-1} (based on induction of hemangiosarcomas and hemangiomas in mice (Hamada, 1993b)), resulted in oncogenic risk values greater than the health protective standard of 10^{-6} in all adult populations analyzed.

C. TECHNICAL AND PRODUCT FORMULATIONS

Carbaryl is registered in California as an insecticide, acaracide and molluscicide. Additional registrations as a fungicide, herbicide, miticide, disinfectant and repellent are currently inactive in the state. As of the last reported update of the DPR product database 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% (±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 [a]	Pounds applied [a]	Agricultural fraction(%) [b]
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

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

[b] "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, 2014).

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.)

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

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

G. ENVIRONMENTAL FATE

The following summary was extracted from DPR Environmental Monitoring reports by Xu and by Gunasekara. These reports can be found at http://www.cdpr.ca.gov/docs/emon/pubs/envfate.htm

Air. Carbaryl has a low vapor pressure (1.17 x 10^{-6} mm Hg 25°C) and low Henry's Law constant (2.74x10^{-9} atm m^3 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_2 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 adsorbtion coefficient (K_{oc} = 100 - 600, with the precise value dependent on soil type), octanol / water partitioning (log K_{ow} = 1.85 - 2.36) and low water solubility (113 ppm at 22°C - though see footnote #1, Table II-2). Soil binding is also favored under acidic conditions. Mud has been shown to enhance the removal of carbaryl from aqueous systems, presumably by preferential binding. Carbaryl tends to be associated with the top 0 - 0.15 meters of soil, showing a dissipation half-life of 0.76 - 10.9 days. Exchangeable cations such as potassium enhance the adsorption of carbaryl to soils. Soil adsorption capacity was also enhanced by the presence of organic matter.

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

III. TOXICOLOGY PROFILE

A. PHARMACOKINETICS

1. Overview

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_2 (detectable when carbaryl is labeled in the carbonyl or N-methyl carbon, but not when labeled in the naphthalene ring). The recovery kinetics in other laboratory species examined (mouse, guinea pig, sheep) appeared generally similar, though there were significant technical problems with these latter studies, which were conducted in the 1960s, used very few animals and left large fractions of the administered dose unanalyzed. A possible exception to the rat model was the dog, where approximately equal fractions were excreted in the 24-hr urine and feces, though these data suffered from similar problems.

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

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

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

Table III-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 gluathione via the mercapturic acid pathway; (2) carbamate hydrolysis to form 1-naphthol; and (3) oxidation of the N-methyl moiety. Metabolites were conjugated with sulfate or glucuronic acid. The proposed metabolic scheme appears in Figure 1.

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

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[1] This risk characterization document contains technical references to the acceptability, non-acceptability or supplemental quality of the studies used to gauge risk. These designations refer to each study's status with regard to guidelines established through the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). In this context, a "supplemental" designation indicates that the work was not done using those guidelines. However, while the FIFRA designation was an important consideration in the risk evaluation, the usefulness of a study was ultimately independent of that designation.

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

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

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

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

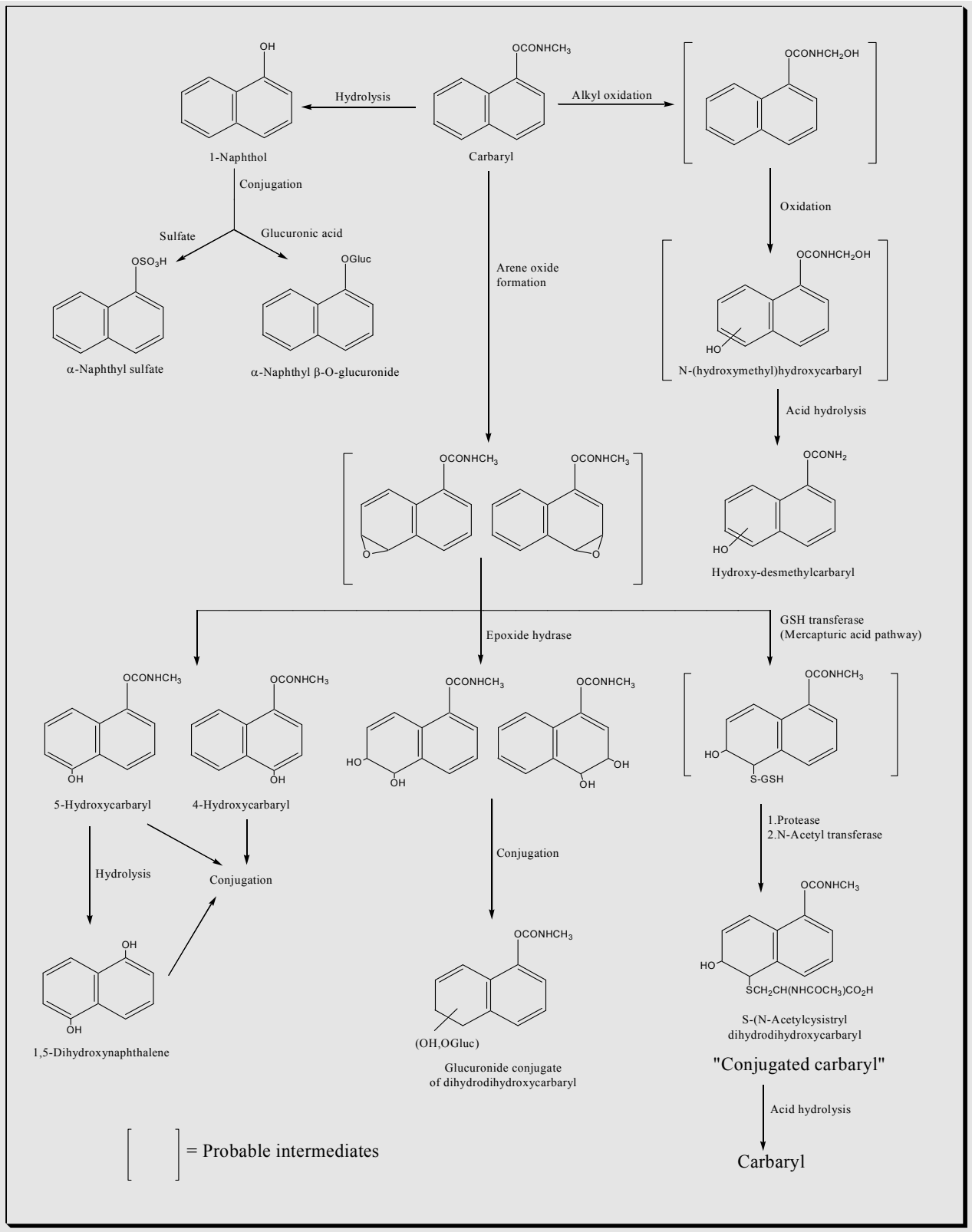
Table III-1b. Excretion time course for ^{14}C-carbaryl administered intravenously (Group A) and by oral gavage (Groups B-D); female HSD:SD rats (Struble *et al.,* 1994)

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

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

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

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



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

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

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

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

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

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Similar to the Totis (1997) study in rats, Valles (1999) initiated a study in CD_1 mice to "investigate the contribution of metabolism to the mechanisms that resulted in the appearance of an increased incidence of tumours during the final year of a chronic dietary feeding study [in mice] at the dose level of 1000 and 8000 ppm". Males (10/dose) were fed diets containing 0, 10, 100, 1000 or 8000 ppm carbaryl for 14 days, followed by a single gavage dose of 50 mg/kg ^{14}C-carbaryl (labeled at the naphthalene-1 position) on day 15. Assuming a food consumption rate of ~6 g/day and body weights of about 0.03 kg, these doses corresponded to carbaryl doses of approximately 0, 2, 20, 200 and 1600 mg/kg/day. Urine and feces were collected at 24-hr intervals for a total of 168 hr following dosing, after which the animals were sacrificed.

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

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

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

This study was deemed supplemental.

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

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

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

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

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

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

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

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

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

This study was considered to be supplemental.

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The following three paragraphs summarize three older metabolism studies from Knaak *et al.* (1965, 1967, 1968) in several species. Knaak's rat data largely support Struble (1994), though it

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

appears that the dog may excrete a higher proportion of the naphthyl residues in the fecal fraction and may not produce certain metabolites in the urine (Knaak, 1967). However, Knaak's studies used low animal numbers and did not analyze the metabolites in large fractions of the total dose, particularly in the feces.

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

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

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

Fluorescence chromatograms were conducted on 24-hr pooled urine samples from men exposed to carbaryl dust in a packaging operation at a Union Carbide plant (though the number

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

of men was not stated). The only detectable metabolites were 1-naphthyl glucuronide (~25 µg/ml) and 1-naphthyl sulfate (~5 µg/ml). These data demonstrated that humans can hydrolyze and conjugate carbaryl. The apparent absence of other metabolites may be a function of the sensitivity or timing of the fluorescence assay.

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

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

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

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

A spectrum of metabolic products resulting both from hydroxylation and hydrolysis of carbaryl were noted in the 24-hr urine samples of all species tested. In the pig, two major metabolites possessing the intact carbamate structure (*i.e.*, C-O-C(O)-N-C), including 1-naphthyl methylimidocarbonate O-glucuronide (compound D: 38-46% of total ^{14}C recovered from the column) and 4-(methylcarbamoyloxy)-1-naphthyl glucuronide (compound F:15-16%), were recovered. In addition, unidentified neutrals (referred to as compound A, probably including parental carbaryl and naphthol: 10-23%) and one hydrolysis product, 1-naphthyl glucuronide (compound G: 5.5%) were also detected. The ewe excreted five intact carbamates: compounds D (26-42%), F (13-24%), H (4-(methylcarbamoyloxy)-1-naphthyl sulfate: 4-13%), and two unidentified intacts not identified in rat, guinea pig, monkey or pig urine (compound J: 6.9% and compound K: 3-9%). Also identified in ewe urine were neutral compound A (3-14%), and two hydrolysis products, compounds G (11.9%) and I (1-naphthyl sulfate: 25.2%). The monkey excreted three intact carbamates, including compounds D (16-18%), F (31-38%) and H

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

(14-26%), in addition to neutral compound A (16-17%). Virtually no hydrolyzed metabolites (*i.e.*, compounds G or I) were excreted in monkey urine.

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

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

This study was considered to be supplemental.

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

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

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

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

This open literature article was considered to be supplemental.

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

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

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

B. ACUTE TOXICITY (including ACUTE NEUROTOXICITY)

1. Overview

The acute toxicity of carbaryl results from its ability to carbamylate, and thus inhibit, acetyl cholinesterase (AChE) at synapses and neuromuscular junctions. Resulting local accumulations of acetylcholine (ACh) generate cholinergic effects, including tremors, sluggishness, epigastric pain, blurred vision, nausea, sweating, lassitude, salivation, piloerection and lacrimation. According to Baron (1991), only one human death, a suicide, was unambiguously tied to carbaryl ingestion at 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.

The lower LD_{50} reported for the intraperitoneal route than for the oral route in rodent studies implies that hepatic (or possibly gastrointestinal) metabolism and excretion plays an important mediating role in the organismal response to carbaryl. The critical LOEL for oral toxicity was 1 mg/kg ("weak effect") or 10 mg/kg ("strong effect"), based on cholinergic effects in rats at those doses in the developmental neurotoxicity study of Robinson and Broxup (1997) and summarized below in section III.H. Benchmark dose analysis was used to arrive at an oral LED_{10} 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^3, based on inhibition of brain cholinesterase activity at that dose in rats after a 3-hr exposure (Weinberg, 2008). Benchmark dose analysis was used to arrive at an inhalation LED_{10} of 9.81 (14.15) mg/m^3, equivalent to an internal dose of 1.18 (1.70) mg/kg assuming a default breathing rate of 0.96 m^3/kg/day.

A summary of acute LD_{50}, LC_{50} and primary eye and skin irritation data appear below in Tables III-2a and III-2b. A summary of NOEL, LED and LOEL values for acute toxicity studies on carbaryl appear below in Table III-6.

2. Human exposures

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

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

was 18 per minute. During the entire course of poisoning, no miosis, excess lacrimation or salivation, or rales were observed. Definite improvement, including some increase in strength, appeared a little less than 3 hr after the onset of symptoms, and recovery was nearly complete 4 hr after onset."

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

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

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

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

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

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

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

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Dickoff *et al.* (1987) described a case in which a 23-year old man purposely swallowed 100 ml of Ortho-Liquid-Sevin (27% carbaryl in water), equivalent to a dose of ~500 mg/kg body weight.

The same individual had consumed an unknown quantity of boric acid on the same day and a "small amount" of dicumarol rat poison the day before. The observed effects were attributed to the very high carbaryl exposure (*i.e.*, comparable to the rodent LD_{50}). The following observations were recorded (this list is largely quoted from the manuscript):

(1) found comatose 3 hr post carbaryl ingestion;

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

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

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

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

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

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

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

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

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

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

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

3. Laboratory animal studies

a. LD_{50}, LC_{50} and primary eye and skin irritation

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

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

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

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

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

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

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

b. Full acute toxicity studies

Moser (2007; the data also appear in Moser *et al.*, 2010, which is available in the open literature) investigated the effects of a single gavage dose of carbaryl on cholinesterase activity (brain and RBC) and motor activity in adult (92 days) and young (postnatal [pnd] days 11 and 17) male Long-Evans hooded rats. The doses were 0 (corn oil vehicle), 3, 7.5, 15 and 30 mg/kg body weight. ChE assays from tissue samples were performed 40 minutes after dosing, with special care taken to minimize carbaryl dissociation from the enzyme during the radiometric procedure. Motor activity, a measure of neurotoxicity, was gauged in the pnd17 rats only. This was done 15 minutes after dosing using a single 20-minute activity session conducted in a

figure-eight chamber. The results of the pnd17 motor activity assays were compared to previously collected data in adult animals. The number of animals examined per dose was based on the expected variability of the ChE and neurotoxicity endpoints in young and adult rats. Thus for adults, 6 animals per dose were used, so that a statistically significant 10% change in enzyme activity could be detected. Eight animals per dose were used for the pnd11 animals due to the higher enzyme variability at the younger age. For the pnd17 animals, 10 animals per dose were tested because the neurotoxicity assays were known to be more variable than the ChE assays - thus a statistically significant 30% change in motor activity would be detected by this number of animals.

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

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

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

Table III-3. Brain and RBC cholinesterase activities, motor activities and ED_{10} and LED_{10} values following a single gavage dose in male Long-Evans rats (Moser, 2007 and Moser *et al.*, 2010)

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

Abbreviation: nd, not determined

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

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

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

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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 ≥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 ≥50 mg/kg. With the exception of staining, decreased activity and weakness, many of the signs were no longer observed 1 day after dosing. Weight losses were observed at all doses >10 mg/kg.

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

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

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

Brain cholinesterase activities showed marked inhibition at 0.5 hr (activities were 46%**, 23%** and 18%** of concurrent controls at increasing doses in males, 54%**, 24%** and 22%** of controls in females) and 1 hr (males 68%**, 25%** and 22%** of controls; females: 64%**, 23%** and 16%**; $p<0.01$, Dunnett's test), declining steadily thereafter, though inhibition was still present after 24 hr at 125 mg/kg in both sexes at the high dose (77% and 65%** of controls). A similar pattern was evident for whole blood cholinesterase activities, though the extent of the inhibition was somewhat less than for brain. Plasma cholinesterase activities were

markedly inhibited at 0.5 and 1 hr (males, 0.5 hr: 64%*, 24%** and 19%** of controls; females, 0.5 hr: 62%, 29%* and 31%*; males, 1 hr: 68%*, 27%** and 17%**; females, 1 hr: 71%*, 25%** and 11%). Substantial recovery had occurred by 24 hr except at the high dose where male and female activities were 59%* and 46%** of controls.

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

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

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

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

This study was deemed supplemental.

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

There were no deaths. Clinical signs, noted in females at 50 mg/kg and in both sexes at 125 mg/kg, were dominated by observations of fur staining and ocular signs. Mid and high dose males exhibited reduced body weight gains during the first week, but appeared to compensate during the second week (mean male weight gains in grams, week 1, at ascending doses: 41.8, 40.7, 32.4*, 13.0**; week 2: 35.6, 37.5, 34.1, 41.3; *, **$p<0.05$, 0.01). High dose females exhibited reduced body weight gains during the first week, but also appeared to compensate

during the second week (mean female weight gains in grams, week 1: 16.5, 16.5, 13.0, 9.6; week 2: 13.3, 15.2, 13.5, 22.9*; *p<0.05). Food intake was decreased during week 1 (mean male intake in grams/rat at ascending doses, week 1: 183.9, 203.7, 181.5, 148.4**; week 2: 204.8, 214.3, 195.9, 188.1; mean female intake, week 1: 132.5, 138.9, 127.2, 109.3**; week 2: 135.6, 145.6, 137.5, 141.7; **, p<0.01).

FOB analysis revealed effects in both sexes at 50 and 125 mg/kg on day 0. In many instances dose responsiveness was evident with respect to severity and incidence, with most observations achieving statistical significance. These included ⇑ incidence of salivation and / or wet muzzle, ⇑ incidence of tremors, ataxic gait / overall gait incapacity, ⇓ locomotor activity, arousal and # of rears, ⇑ positional passivity, ⇓ extensor thrust, tail and toe pinch responses, impaired visual placing response, ⇓ urination and defecation in males, ⇓ vocalization upon cage removal in females, ⇑ auricular startle response (high dose), ⇑ incidence of males lying on ventral surface (high dose), ⇓ fore and hind grip strength (high dose), ⇑ hindlimb splay (males, high dose), and ⇓ body temperature. These effects had largely abated by days 7 and 14.

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

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

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

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

	Males				Females			
	0 mg/kg	**10 mg/kg**	**50 mg/kg**	**125 mg/kg**	**0 mg/kg**	**10 mg/kg**	**50 mg/kg**	**125 mg/kg**
Pre-study	255.3±87.6	303.2±92.8	294.1±104.8	252.0±85.3	298.8±112.8	272.4±66.6	316.9±140.3	250.5±97.9
Day 0	221.7±51.3	177.2±59*** (80%) [b]	53.7±32.4*** (24%)	35.8±23.7*** (16%)	393.8±127.6	314.3±101.6# (80%)	36.9±31.4### (9%)	47.8±65.3### (12%)
Day 7	217.9±57.0	281.3±57.7 (129%)	322.8±139.0 (148%)	193.3±88.1 (89%)	425.9±107.2	391.8±143.6 (92%)	503.8±161.7 (118%)	298.4±138.4 (70%)
Day 14	246.3±80.1	293.6±98.7 (119%)	347.7±153.6 (141%)	227.5±94.5 (92%)	452.9±132.2	406.3±115.5 (90%)	451.1±131.4 (100%)	362.3±158.9 (80%)

***: $p<0.001$, t-test, linear constructed variable
#, ###: $p<0.05$, 0.001, Wilcoxon, linear constructed variable
[a] Motor activity was determined in a "Figure 8 enclosure" based on interruption of a light beam. The 1-hr sessions were recorded by a microcomputer in 30 successive 2-min intervals. The mean number of counts/hr ± the standard deviation is expressed in this table.
[b] Numbers in parentheses represent percent of concurrent controls.

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

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

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

There were no deaths during the 2-week course of the study. A 27 g loss of body weight at 125 mg/kg between days 0 and 1 resulted in significantly lower body weights on days 1 and 7 (9.5% and 6%, respectively; $p<0.01$). Body weight effects were not apparent at the other doses.

FOB analysis on day 0 showed the following effects at 125 mg/kg: tremors; gait incapacity; salivation; miosis; decreased locomotor activity, arousal and defecation; abnormal responses to sensory tests and others. Day 0 FOB effects at 40 mg/kg included tremors; salivation; and decreased locomotor activity, arousal, toe/tail pinch and defecation. Defecation was also statistically reduced at 12.5 mg/kg on day 0 (# of fecal "boli" during a 2-min period in the day 0 FOB: 1.9±1.4, 0.8±1.0*, 0.8±0.7*, 0.0±0.0***; *p<0.05; ***p<0.001). However, these data were very hard to interpret in view of the short observation period. Forelimb and hindlimb grip strength were reduced on day 0 at 125 mg/kg, with hindlimb grip strength also reduced at 40 mg/kg. Foot splay was significantly increased in both dose groups on Day 0. Body temperature was reduced on day 0 for all dose groups (°C): 38.0±0.33, 37.3±0.99*, 34.9±0.53** and 34.3±0.78** (*p<0.05; **p<0.01). There was, however, concern that the control body temperatures were inappropriately high, casting doubt on the apparent temperature lowering response at 12.5 mg/kg. Group mean total activity counts were lower at 40 and 125 mg/kg (209, 207, 43.3*** and 23.7***; ***p<0.001). Total activity counts were still statistically depressed on day 1 at the high dose, though no significant differences remained by days 7 and 14.

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

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In an open literature study, Moser *et al.* (1988) used the functional observational battery (FOB) to discern probable neurotoxic responses in Long-Evans hooded rats, 10/sex/dose, to a single intraperitoneal dose of carbaryl . The carbaryl doses were 0 (vehicle control: 5% ethanol-5% Emulphor in saline), 3, 10 and 30 mg/kg. The effects of chlordimeform were also examined, though those results will not be summarized here. The FOB tests were run prior to dosing and at 0.5, 3, 24 and 48 hours post dose. The following parameters were examined: posture, palpebral closure, presence or absence of writhing, circling, biting or vocalizations, ease of removal / handling, observable signs (exopthalmus, crustiness around the eyes, piloerection, bite marks on tail or paws, missing toenails, body tone and emaciation), "cart top" measurements (latency to first step, number of rears [supported and unsupported], grooming episodes, gait characteristics, arousal level, number of fecal boluses and urine pools), reflex testing (responses to approach of a pencil, a touch to the rump, finger snap, tail pinch), pupil contraction to light, extensor thrust, limb rotation, degree of catalepsy, righting reflex, grip strength, foot splay, body weight and rectal temperature. The entire exam required 6-8 minutes per rat.

Effects noted at both 10 and 30 mg/kg included the following (in both sexes unless otherwise noted): decreased rearing, decreased arousal, home cage posture alteration, decreased removal difficulty (males only), convulsions, increased urination (females at high dose only), home cage palpebral closure (females at high dose only), pupil response, righting reflex, decreased approach response (females at high dose only), decreased finger snap response (males at high dose only), decreased touch response, decreased tail pinch response, chewing motions, decreased rectal temperature and decreased body weight (females at high dose only). Effects noted at 30 mg/kg only included increased latency to first step (males only), abnormal fur appearance, decreased defecation, salivation, piloerection (males only), affected gait, decreased forelimb grip strength (females only), palpebral closure at handling (males only), generalized tremors and catalepsy. For those parameters for which time data were reported, the most severe responses were noted at 0.5 and 3 hr post dose. There was limited evidence for a

slight increase in unsupported rears in males at 3 mg/kg, though dose dependence was not in evidence. In the absence of other signs at that dose, this particular observation was considered inadequate to determine a LOEL.

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

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

All animals survived the exposure period. Necropsies and brain weight determinations were unremarkable. RBC and brain cholinesterase activities in the first cohort were reduced in a statistically significant, dose-dependent manner for both sexes in all exposure groups (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^3 (1.2 mg/kg for the 3-hr exposure using the default breathing rate of 0.96 m^3/kg) was assigned based on the inhibition of brain cholinesterase in females at the low dose in cohort #2. Benchmark dose analysis of these data using the power algorithm (power unrestriced) resulted in LED_{10} (ED_{10}) values of 9.81 (14.15) mg/m^3, 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^3) - males				Carbaryl (mg/m^3) - females			
	0	**63**	**121**	**247**	**0**	**63**	**121**	**247**
RBC, U/L **% of control**	3708±856	2317±182** 62.5%	1625±35** 43.8%	1040±484** 28.0%	4067±638	1551±620** 38.1%	955±446** 23.5%	831±460** 20.4%
Brain, U/L **% of control**	47391±1293	36116±1298** 76.2%	32512±3054** 68.6%	23302±4390** 49.2%	45767±2342	31991±4565** 69.9%	23563±4773** 51.5%	19861±2462** 43.4%

**p<0.01

Cohort 2:

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

**p<0.01

Table III-6. NOEL, LED and LOEL values for acute toxicity studies on carbaryl

Species, strain	Study type, exposure regimen	Effects at LOEL	NOEL or LED	LOEL	Reference [f]
rat, Long-Evans (♂ only)	oral gavage, single dose	⇓ brain & RBC ChE activity	1.1 mg/kg (LED_{10}) for pnd 11 rats [a]	3 mg/kg	Moser (2007) *Supplemental*
rat, Sprague-Dawley	oral gavage, single dose	cholinergic signs	10 mg/kg [b]	50 mg/kg	Brooks & Broxup (1995a) *Supplemental*
rat, Sprague-Dawley	oral gavage, single dose	⇓ brain, RBC & plasma ChE activity	nd	10 mg/kg (ldt)	Brooks & Broxup (1995b) *Supplemental*
rat, Sprague-Dawley	oral gavage, single dose	⇓ brain, RBC & plasma ChE activity	nd	10 mg/kg (ldt)	Brooks & Broxup (1995c) *Supplemental*
rat, Sprague-Dawley	oral gavage, single dose	⇓ motor activity counts	nd	10 mg/kg (ldt)	Brooks *et al.* (1995) *Supplemental*
rat, Sprague-Dawley (♂ only)	oral gavage, single dose	cholinergic signs, ⇓ body weight gain	25 mg/kg	80 mg/kg	Beyrouty (1992a) *Supplemental*
rat, Sprague-Dawley (♂ only)	oral gavage, single dose	FOB signs and ⇓ body temperature	footnote "c" [c]	40 mg/kg	Beyrouty (1992b) *Supplemental*
rat, Sprague-Dawley	oral gavage, daily, gd 6 - ppd 10 [d]	⇓ body weight gain, FOB signs, ⇓ RBC & brain ChE	**1 mg/kg [d]**	10 mg/kg	Robinson & Broxup (1997)
rat, Long-Evans	intraperitoneal, single dose	FOB signs	3 mg/kg [e]	10 mg/kg [e]	Moser *et al.* (1988) *Supplemental*
rat, Sprague-Dawley	inhalation, single 3-hr exposure	⇓ brain ChE	9.81 mg/m^3 (1.18 mg/kg) (LED_{10} in ♀)	10 mg/m^3 (1.2 mg/kg)	Weinberg (2008) *Supplemental*

Abbreviations: pnd, postnatal day; nd, not determined; ldt, lowest dose tested; FOB, functional observational battery; gd, gestation day; ppd, post partum day

[a] The LED_{10} was derived by the USEPA (USEPA, 2007a).

[b] This NOEL was considered to be conditional, due to low animal numbers and limited observational time.

[c] There were possible effects on defecation and body temperature at 12.5 mg/kg, though they were not considered strong enough to establish a LOEL.

[d] The NOEL of 1 mg/kg was used to evaluate acute risk in this document. Possible signs at 1 mg/kg (slight hypotonic gait and slight tremors) were considered insufficient to constitute a critical LOEL determination

for regulatory purposes in this risk assessment. However, a LED_{10} of 0.25 mg/kg was calculated based on the incidence of slight hypotonic gait over the dose range. This was offered as an alternative critical POD in DPR's earlier dietary risk assessment on carbaryl (DPR, 2010). For details, see the text passages in sections III.H., IV.A.1. and V.A.1.a. Only the maternal data from that study are relevant to this summary table. This study is described in section III.H. below.

[e] 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.

[f] The designation "Supplemental" indicates that the study was not conducted according to FIFRA guidelines; however, such studies were reviewed and contribute to the general acute toxicologic picture of the chemical.

C. SUBCHRONIC TOXICITY (including NEUROTOXICITY)

1. Overview

Three subchronic oral studies, in mice, rats and dogs, were available for analysis. As none of these studies were conducted according to FIFRA guidelines, each was considered supplemental. Neither the mouse nor the dog studies showed clear adverse effects. However, neurotoxic effects were observed in the rat with respect to maze performance, EEG readings and brain cholinesterase activities. As the changes in maze performance were manifest soon after the advent of dietary exposure, they were consistent with an acute effect. Three 4-week repeat dose dermal studies using 99.49%, 44.82% and 80.07% formulations were also conducted. Carbaryl inhibited both RBC and brain cholinesterases at a LOEL dose of 50 mg/kg/day (99.49% and 80.07% formulations). Except for possible weight decrements and local irritation noted with the 99.49% formulation, there were no other effects.

Subchronic NOELs and LOELs are summarized in Table III-10.

2. Laboratory animal studies

a. Oral exposure

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

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

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

This study was considered to be supplemental.

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Rats. Desi *et al.* (1974) examined the neurotoxicologic effects in male Wistar rats (R strain) of daily dietary exposure to carbaryl. The exposure period was up to 50 days. Another carbamate, Arprocarb, was also tested, though those data will not be summarized here. The stated aim of the study was to provide information on (1) whether these compounds can be

used safely over extended periods and (2) what the relationship is between cholinesterase inhibition and the observed neurotoxicity.

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

During the first 2 weeks, both dose groups performed notably better in the mazes than controls, achieving the goal 10-12 seconds faster at day 11 ($p<0.05$, for both groups). However, by day 25, there were no clear differences between treated animals and controls, a situation that was maintained through the end of the study at day 50. A similar observation was made for error frequency - while significantly fewer errors were made in both dose groups on day 11 ($p<0.001$), all groups (including controls) declined to a minimum error frequency around day 22-24, followed by a general increase in error frequency in all groups after that point with no clear difference between treated animals and controls.

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

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

Animals exposed to 20 mg/kg/day carbaryl for 50 days registered statistically lower cholinesterase activities in all brain regions examined. Thus cortex, white matter, brain stem and cerebellum exhibited 60.5%, 85.8%, 56.3% and 60.9% of control activities. Animals exposed to 10 mg/kg/day did not evidence statistically significant changes (though 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 µM/ml, day 14: 8.9, 7.3*, 8.1, 6.9*; *$p \leq 0.05$). As there was no clear dose responsiveness, no sign of an effect in females, no statistically significant effects at day 32, and the "inhibition" characteristics were roughly shared by the same animals when measured on three separate occasions *before* the commencement of dosing (*eg.*, plasma ChE activities on day -8 were 9.1, 7.7, 8.6 and 8.3 µM/ml), the depressions on day 14 were not sufficiently clear to be considered a definite function of carbaryl exposure. Even so, the possibility of inhibition, particularly at the high dose, was not definitively excluded.

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

b. Dermal exposure

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

All animals survived the treatment. There were no behavioral or morphologic signs attributed to carbaryl exposure. Mean body weight gain for high dose males was statistically lower than controls for the day 5-12 period (weight gains at ascending doses, males, day 5-12: 33, 35, 34, 24* g; *$p \leq 0.05$). Decreased (though 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 (♂ & ♀) and 12 (♂ @ 100 mg/kg/day only, ♀ @ 50 & 100 mg/kg/day). On day 19, statistically significant inhibition was registered at 100 mg/kg/day in females only, while on day 26 no inhibition was noted in either sex (Table III-7). 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^2 (assuming a body surface area for a 200 g rat of 325 cm^2, as in Harkness and Wagner, 1983), based on the atonia noted at 100 mg/kg/day.

This study was considered to be supplemental.

Table III-7. RBC and brain cholinesterase activities in a 4-wk carbaryl repeat-dose dermal study in Sprague-Dawley rats (Austin, 2002a)

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

*p<0.05

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

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

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

cholinesterase data.

This study was considered supplemental.

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

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

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

This study was considered to be supplemental.

D. CHRONIC TOXICITY AND ONCOGENICITY

1. Overview

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

Chronic NOELs and LOELs are summarized in Table III-11.

2. Laboratory animal studies

Rats. Hamada (1993a) exposed Sprague-Dawley rats to dietary carbaryl (purity, 99%) for 2 years. The doses were 0, 250, 1500 and 7500 ppm, corresponding to mean systemic doses of 0, 10.0, 60.2 and 349.5 mg/kg/day in males and 0, 12.6, 78.6 and 484.6 mg/kg/day in females. There were 90 rats/sex in the control and high dose groups, and 80 rats/sex in the low and mid dose groups. Examined parameters included mortality, clinical signs, body weights, food consumption, hematology, blood chemistry, ophthalmology, gross pathology, histopathology and organ weights. Interim sacrifices on 10/sex/dose were carried out at 26 and 52 weeks. Another 10/sex from the control and high dose groups were reestablished on basal diet for 4 weeks between weeks 53 and 57. Finally, 10 rats/sex/group were subjected to clinical laboratory exams after 78 and 104 weeks and sacrificed at study termination with the remaining animals.

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

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

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

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

controls. The body weight and food consumption effects for the main study animals are summarized in Table III-8a.

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-8b.

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-8c and discussed in the

following paragraphs.

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

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

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

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

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

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

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

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

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

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

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

The NOEL for non-oncogenic systemic effects was established at 250 ppm (10.0-12.6 mg/kg/day) based on (1) the inhibition of brain cholinesterase at 1500 ppm (60.2-78.6 mg/kg/day) and (2) the 18% overall inhibition in female weight gain at 1500 ppm over the 2-year period. Based on the evidence for neoplasia in the urinary bladder, liver and thyroid, carbaryl is clearly carcinogenic in rats. Tumor induction was seen mainly at the high dose, which---based on the large body weight decrements, clinical signs and statistically significant plasma, RBC and brain cholinesterase inhibition---exceeded the 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-8a. 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 [a]				Carbaryl dose (ppm), females [a]			
	0	250	1500	7500	0	250	1500	7500
Body weight (g) **Week 1**	235	234	236	235	179	176	178	175*
Week 4	385	376*	368*	299*	244	242	236*	201*
Week 17	586	569*	569*	442*	331	327	315*	243*
Week 21	620	601*	595*	457*	344	342	326*	245*
Week 53	724	716	708	526*	431	425	410*	264*
Week 79	745	736	718	523	470	463	437	281
Week 105	717	692	677	463*	510	501	450*	278*
Food consumption (g) weeks 1-102	6568	6385	6373	5407*	4918	4938	4955	4144*

* $p<0.05$

[a] Mean systemic doses: 0, 10.0, 60.2 and 349.5 mg/kg/day in males and 0, 12.6, 78.6 and 484.6 mg/kg/day in females

Table III-8b. Cholinesterase activities for Sprague-Dawley rats exposed to dietary carbaryl for 2 years (Hamada, 1993a)

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

* $p<0.05$

[a] Mean systemic doses: 0, 10.0, 60.2 and 349.5 mg/kg/day in males and 0, 12.6, 78.6 and 484.6 mg/kg/day in females.

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

[c] n/a, not applicable

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

Table III-8c. Neoplastic and non-neoplastic changes in Sprague-Dawley rats exposed over a 2-yr period to dietary carbaryl (Hamada, 1993a)

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

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

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

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

[a] Mean systemic doses: 0, 10.0, 60.2 and 349.5 mg/kg/day in males and 0, 12.6, 78.6 and 484.6 mg/kg/day in females.
[b] T, terminal sacrifice; U, unscheduled deaths
[c] B, benign; M, malignant
[d] ic, intracytoplasmic; n/a, not applicable
[e] While incidence rates for sciatic nerve degeneration were similar among dose groups, the severity of this parameter increased at the high dose in both sexes (see text).
[f] Sum of "at risk" urinary bladder transitional cell papillomas and transitional cell carcinomas.

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

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

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

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

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

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

Mice. Hamada (1993b) exposed 80 CD-1 mice/sex/dose to dietary carbaryl (purity, 99.3%) for two years. The doses were 0, 100, 1000 and 8000 ppm, corresponding to average internal 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/µl) and 105 (845, 957, 1185, 1568* th/µl), as were lymphocyte, corrected white blood cell and eosinophil counts at week 53. Because of their consistency (RBC parameters) and magnitude (platelet counts in females), these changes were considered due to carbaryl exposure, though a mechanism is not known.

Statistically significant reductions in RBC cholinesterase activities were recorded in mid and high dose males at week 53 (7.3, 7.0, 5.6*, 5.1* µmol/ml). Statistically significant reductions in brain cholinesterase activities were noted for mid and high dose males and females at week 53 (♂: 86.0, 81.3, 70.7*, 36.9*; ♀: 84.1, 81.1, 73.5*, 44.6* µmol/g) and for mid and high dose males and high dose females at week 105 (♂: 59.9, 59.4, 52.0*, 35.9* µmol/g; ♀: 62.2, 58.7, 55.2, 41.0* µmol/g). The depressed activities at the low dose were possibly carbaryl related, though the small decrement and lack of statistical significance made this unclear. In addition, clinical signs were not apparent at the low dose.

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

Weight differentials were noted at wk 105 in several organ systems at the high dose. These included lung (statistically decreased absolute weight at the high dose and relative weights at the mid and high doses in females), liver / gall bladder (statistically increased relative weights at the high dose, both genders) and kidney (statistically increased relative weights at the high dose, both genders). Similar changes were evident at the wk 53 interim sacrifice. Absolute and relative high dose ovary weights were statistically suppressed at interim sacrifice, though not at terminal sacrifice. These organ weight changes are summarized in Table III-9a.

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-9b). Increased extramedullary hematopoiesis was noted among high dose interim sacrifices, and exhibited slightly increased severity at terminal sacrifice (though incidence was similar to controls). The report suggests that both of these parameters reflected an increased splenic turnover of red blood cells with secondary extramedullary hematopoiesis. The RBC-related hematologic changes noted above may also be related to these splenic effects.

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

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

Oncogenic observations are summarized in the following paragraphs. Carbaryl had clear oncogenic effects, as noted below in vascular tissue (many organs), kidney and liver.

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

Vascular tissue: An increased incidence of vascular neoplasms, identified as hemangiomas and hemangiosarcomas, was noted in males at all doses and in females at the high dose. Statistical significance in pairwise comparisons was evident by the mid dose in males. The increase occurred among unscheduled deaths and terminal sacrifices, but not among the interim sacrifices (where the incidence was zero), suggesting that the tumors developed during the second year of the study (recognizing, of course, that the low number of interim sacrifices may preclude positive observations). In fact, no hemangiosarcoma or hemangioma was detected before week 72. It was thus assumed that it took at least one year for these lesions to develop to the point of detection. The animals that died prior to one year were not considered to be "at risk" and were not included in the potency calculations. The final total incidence rate in males (*i.e.*, the total number of tumor-bearing animals, understanding that multiple valscular neoplasms were present in some animals) was 2/66, 6/66, 10/69* and 10/68* at increasing doses (*p<0.05; see footnote d, Table III-9c). 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 into Table III-9c 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-9a. Effects of carbaryl on organ weights; 2 year CD-1 mouse study (Hamada, 1993b)

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

* $p<0.05$

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

Table III-9b. Non-neoplastic changes in CD-1 mice exposed over a 2-yr period to dietary carbaryl (Hamada, 1993b)

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

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

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

Table III-9c. Neoplastic changes in CD-1 mice exposed over a 2-yr period to dietary carbaryl (Hamada, 1993b)

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

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

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

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

This study was considered to be supplemental.

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

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

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

This study is considered to be supplemental.

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

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

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

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

This study was considered to be supplemental.

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The following two open literature studies examine the question of whether carbaryl can modulate tumor production in mice in the context of stimulation by other carcinogens. The first, by Triolo *et al.* (1982), 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±0.0 to 1.2±2.2; Expt. #2 from 1.1±0.0 to 1.3±0.3). However, in the only experiment conducted in the presence of BP, carbaryl increased the percentage of mice with tumors from 88% to 100% (not statistically significant) and the number of tumors per mouse from 3.7±0.6 to 5.7±1.4 ($p<0.05$). Assay of BPH activity in non-BP-treated animals showed no statistically significant effects in liver or lung. In the presence of BP, a statistically significant increase in BPH activity, from 3.06±0.14 pm/mg protein to 3.86±0.11 pm/mg protein ($p<0.025$), was noted in the lung, but not in the liver. The authors speculated that the increased lung enzyme activity may be mechanistically related to the increased tumor production in the same organ, though this will require further experimental verification.

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

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

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

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

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

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

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

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

Cholinesterase activities were suppressed at all time points, often by statistically significant margins (Table III-10). 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-10. Suppression of cholinesterase activities in beagle dogs by dietary carbaryl; 1-yr study (Hamada, 1987)

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

*$p<0.05$

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

[b] Percent of concurrent control activities.

Table III-11. NOEL and LOEL values for subchronic and chronic toxicity studies on carbaryl

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

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

E. GENOTOXICITY

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 ∝-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-12. 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-12. Genotoxic effects of carbaryl

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

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

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

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

F. REPRODUCTIVE TOXICITY

1. Overview

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

In light of these mixed results from laboratory animal studies, an early epidemiologic investigation of testicular function among carbaryl-exposed factory workers was undertaken (Wyrobek *et al.*,1981). This study was designed to determine if spermatogenic effects have occurred in 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 napthalene. Possible reproductive effects of carbaryl were also considered in a recent epidemiologic study of male pesticide exposure and pregnancy outcome among farm families in Ontario, Canada (Savitz *et al.,* 1997). In that study, the adjusted odds ratio for miscarriage rose in conjunction with carbaryl exposure, suggesting that exposure of reproductive-aged males could result in clinically manifested toxicity.

These studies are summarized below and in Table III-14.

2. Human epidemiologic studies

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

The carbaryl air concentrations in the facility were not determined for this study, though air sampling data generated by the company's industrial hygiene program provided an indication of the range of values encountered. Thus three samples from the operations area yielded air

concentrations between 0.36 and 14.21 mg/m^3 (mean = 4.9 mg/m^3), 22 samples from the distribution area ranged between 0.03 and 1.8 mg/m^3 (mean = 0.347 mg/m^3), and 36 personal monitoring samples from the same area ranged between 0.0 and 1.8 mg/m^3 (mean = 0.439 mg/m^3).

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

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

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

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

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

Previously exposed workers, defined above as men with an average time since carbaryl-related employment of 6.3±3.9 years (range = 1-12 years), showed a somewhat higher incidence of abnormal sperm than controls, though this achieved statistical significance only when the entire cohort was considered (*i.e.*, when confounders were included). The proportion

of teratospermic men, defined as those with greater than 60% abnormal sperm forms, rose in the exposed population from 4/34 in controls to 9/30 in currently exposed and 5/19 in previously exposed men. When the two latter groups were combined, creating a teratospermic incidence of 14/49, statistical significance was not achieved (p=0.06).

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

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

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

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

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

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

other pregnancy outcome parameters 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 napththalene) and various sperm parameters in humans. Subjects were recruited from a pool of 330 men seeking diagnoses from a Boston infertility clinic. They were primarily white (82%), 36.2±5.5 years, 72% never having smoked. Subjects were excluded if they had highly concentrated or dilute urine samples as determined by creatinine concentrations or specific gravity. The chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol (TCPY) was also measured, though those results will not be discussed here.

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

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

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

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

In a parallel study, Meeker *et al.* (2004b) tracked DNA damage in human sperm as a function of 1-naphthol and TCPY concentrations in the urine (TCPY is a metabolite of chlorpyrifos; as in the previous study, the TCPY results will not be summarized here). DNA damage was assessed

using a modified "comet" assay, which is based on the electrophoretic distances traveled by negatively-charged DNA molecules from single sperm cells toward a positive electrode. These movements form a characteristic comet tail, the shape of which is indicative of toxic damage due to the creation of DNA fragments, etc. Two hundred and sixty subjects were recruited from a pool of 368 men seeking diagnoses in a Boston infertility clinic. They were primarily white (82%), with a mean age of 36.1±5.6 years. 74% of the cohort had never smoked, while 9% were current smokers. Subjects were excluded if they had concentrated or dilute urine samples. This was determined primarily by urinary specific gravity measurements, but also by urinary creatinine concentrations. Of the original subject pool, there were 19 azoospermic men (*i.e.*, semen with no sperm cells) among a total of 74 subjects whose semen was, for various reasons, not analyzed. 1N was determined in a single urinary sample from each subject.

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

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

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

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

hybridization) were performed to detect chromosome aberrations on the X and Y chromosomes and on chromosome 18 using DNA probes specific to the centromeric regions.

No significant differences were noted for semen volume, sperm concentration, sperm number and sperm motility. Morphologic abnormalities did exhibit a statistical increase, with 20.50±6.71% among internal controls, 15.92±7.58% among external controls and 25.25±4.90%** among the carbaryl-exposed group (**p<0.01 compared to external controls). This was due primarily to statistically significant increases in head abnormalities (9.85±5.12% vs. 7.56±3.61% vs. 11.72±4.93%*; p<0.05) and tail abnormalities (8.73±4.10% vs. 6.60±4.78% vs. 10.82±3.09%**).

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

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

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

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

3. Laboratory animal studies

a. Contract laboratory studies

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

histopathology and organ weights.

There were no treatment-related clinical signs in either sex. Precoital and gestational periods were comparable among groups of both generations. Mean estrus cycle length was slightly longer in F_0 females at 1500 ppm (4.77 days) compared with controls (4.59 days), but (1) the effect was not statistically significant, and (2) estrus cycle lengths among F_1 females were similar across dose groups. In light of the human epidemiological studies, the older Russian studies, and those of Pant *et al.* (1995, 1996), all summarized in this section, it is pertinent to mention that there were no effects on F_0 or F_1 parental epididymal sperm counts, motility, morphology, homogenization-resistant spermatid head counts, daily sperm production or efficiency of daily sperm production.

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

Mean pre-breeding body weights were consistently statistically suppressed at 1500 ppm among F_0 and F_1 animals of both sexes. These were largely reflective of statistical decrements in weight gain during the first pre-breeding week of exposure (Table III-13). There was also some indication that weight gains among the 300 ppm animals were suppressed, particularly among F_1 males. Maternal body weight gains were suppressed at 1500 ppm in both the F_0 and F_1 generations, though lactational weight gains appeared little affected, or were increased, by carbaryl exposure. Mean F_1 and F_2 pup body weights tended to be suppressed at 1500 ppm, particularly as the lactational period progressed (Table III-13, pup body weights, postnatal days 0 and 21).

Pup survival measurements indicated possible treatment effects at 300 and 1500 ppm. Though the data were not as statistically robust as the body weight data, statistical linear trends were detected in survival indices for F_1-day 14 pups, F_2-day 4 pups and F_2-day 7 pups (Table III-13). The mean number of live pups / litter, F_1-pnd 4 (precull) did not appear affected. However, the same parameter for the F_2 pups was statistically depressed at the mid and high doses (Table III-13).

Both male and female F_1 pups appeared to sustain statistically significant developmental delays at 1500 ppm. In males this was represented by a delay in the time of preputial separation, while in females it was represented by a delay in vaginal opening (Table III-13). Because the F_2 pups were sacrificed at weaning, similar measurements were not made for them. However, anogenital distance measurements on F_2 pups did not suggest an effect.

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

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

This study was acceptable by FIFRA guidelines.

Table III-13 . Effect of dietary carbaryl on reproductive parameters in CD rats (Tyl *et al.*, 2001)

	Carbaryl concentration in diet (ppm) [a]			
	0	**75**	**300**	**1500**
Body wt. gain, pre-breeding days 0-7 (g)				
F_0 ♂	58.9	58.6	54.6	45.2**
F_0 ♀	24.5	24.5	23.4	17.9***
F_1 ♂	64.6	63.1	58.6*	54.2**
F_1 ♀	40.1	40.4	37.8	36.3*
Body wt. gain, gestation days 0-20 (g)				
F_0 ♀	138.1	130.2	132.0	121.0***
F_1 ♀	140.1	137.2	134.3	115.3***
Body wt. gain, postnatal days 0-21 (g)				
F_0 ♀	9.3	3.3	12.6	14.4
F_1 ♀	8.5	5.7	10.8	15.8
Pup body wts. (g)				
F_1, postnatal day 0	6.34	6.68	6.41	6.09
F_1, postnatal day 21	48.79	49.46	50.73	43.46***
F_2, postnatal day 0	6.27	6.51	6.58	6.00
F_2, postnatal day 21	50.91	52.30	49.68	40.39***
Pup survival index (%)				
F_1, 4-day	98.4	99.1	95.0	98.1
F_1, 7-day	99.7	100.0	99.6	99.3
F_1, 14-day	99.7^{+}	100.0	99.2	95.4
F_2, 4-day	98.3^{++}	98.7	92.0	88.9
F_2, 7-day	100.0^{+}	99.6	96.0	93.0
F_2, 14-day	98.6	99.6	94.8	96.6
Mean live pups / litter (precull)				
F_1, postnatal day 4	13.9	13.3	13.9	14.3
F_2, postnatal day 4	15.4^{++}	13.9	12.7*	12.5**
Pup developmental indicators				
F_1 ♂, day of preputial separation [b]	41.6	41.5	41.7	43.7**
F_1 ♀, day of vaginal opening [b]	30.6	31.3	31.3	32.0**

*, **, ***: p<0.05, 0.01, 0.001
+, ++: p<0.05, 0.01 (trend test)
[a] Calculated carbaryl intakes for the F0 and F1 parental animals were 5-6 mg/kg/day at 75 ppm, 21-36 mg/kg/day at 300 ppm and 92-136 mg/kg/day at 1500 ppm.
[b] Because the F_2 pups were sacrificed at weaning, similar developmental measurements were not made for them.

b. Studies from the open literature - oral gavage exposure

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

section of the RCD.

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

Subchronic. Male and female rats (32/sex/dose) were treated by gavage with 0 and 50 mg/kg/day carbaryl for 50 days. The following effects were noted in exposed animals: (1) 8-10% weight gain decrement, (2) decrements in butyrylcholinesterase (27%) and acetylcholinesterase (41%; the tissue of origin was not stated), (3) significant decrease in adrenal ascorbic acid levels (amount not stated; p<0.001), (4) 40.4% mean decrease in spermatozoa motility, (5) 11.3% mean delay in the estrus cycle, (6) 50% mean prolongation of diestrus, (7) a decrease in the "period of heat" from 1.34 to 1.05 days, (8) increase in the excretion of gonadotropic hypophyseal hormones was reported in immature mice, though neither the causative dose nor the specific hormones was reported, (9) adrenal and liver weights were increased by 20% and 28%, respectively.

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

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

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

Statistically significant body weight gain decrements were detected "throughout the experiment" at 14 and 70 mg/kg/day (p<0.001), but not at 7 mg/kg/day (data not provided).

Blood butyryl- and acetylcholinesterase were inhibited from the first measurement at 3 months.

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

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

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

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

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

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

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Dikshith *et al.* (1976) treated male albino rats (strain not stated, though they originated in a

colony maintained by the Industrial Toxicology Research Centre, Lucknow, India) with carbaryl (99.0% pure) by oral gavage at doses of 0 (1 ml peanut oil) and 200 mg/kg, 3 days/week, for 90 days. There were 7 animals/dose. Histopathologic analysis was conducted on liver, kidney, testes and epididymis. Biochemical analysis was also conducted as follows: liver and testes - succinic dehydrogenase, adenosine triphosphatase, alkaline phosphatase and acid phosphatase; liver - glucose-6-phosphatase; brain and blood - AChE. A further 7 animals/dose were mated with unexposed females after the 90-day exposure period. When the females were deemed pregnant (by examination of vaginal smears for sperm), they were separated and allowed to complete the pregnancy. Litters were evaluated for weight and numbers of pups born. Pups were observed for 10 days *post partum*.

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

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

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

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

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

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

Examination of the testicular slides indicated "an obvious reduction in the number of the cells in the seminiferous tubules, especially in spermatogonia and in spermatozoa" (p. 55.) A

micrograph from a treated and from a control testis seemed to support this statement, though the prevalence of the effect (*i.e.*, the number of animals affected *vs.* controls) was not reported. There also appeared to be a reduction in the number and size of Langerhans islets in the pancreas. Effects on the adrenals were unremarkable.

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

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

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

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

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

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

Testicular glucose-6-P-dehydrogenase (associated with premeiotic germ cells) and sorbitol dehydrogenase (associated with pachytene spermatocyte maturation) were suppressed at the high dose (G6PDH activities at ascending doses: 89.1, 79.1, 26.3* nmol/min/mg protein;

SDH: 3.18, 2.94, 1.63*; $p<0.05$). Testicular lactate dehydrogenase (associated with germline elements of the testes; inversely proportional with sperm maturation) and γ-glutamyl transpeptidase (marker enzyme for Sertoli cell function) were statistically increased at both doses (LDH: 244, 390*, 500* nmol/min/mg; γGT: 23.2, 37.3*, 58.3*).

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

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

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

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

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

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

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

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

Collins *et al.* (1971) studied the reproductive effects of carbaryl in Mongolian gerbils (*Meriones unguiculatus*), comparing the results to a parallel study in Osborne-Mendel rats (data not summarized here). Gerbils were fed diets containing carbaryl (99% pure) at 0, 2000, 4000, 6000 or 10,000 ppm for 100 days starting at weaning. Forty control pairs were then mated, along with 30 pairs for each dose group except for the high dose, which had 18. Non-survival of high dose F_{2b} males made it necessary to reuse F_{2a} males to generate the F_{3b} generation. Litters were

observed on the day of birth to determine the number of stillborn and liveborn young and for abnormalities. They were observed again on *post partum* day (ppd) 4 for number and condition of the living pups. At weaning, F_{3a} and F_{3b} animals from the 0 and 6000 ppm groups were preserved for histopathology.

Impairment of fertility was evident at the high dose, becoming certain with the F_2 generation. Statistically significant effects at other doses were less clearly related to exposure [9]. 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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[9] Data for the parameters discussed in the summary of the Collins *et al.* study in gerbils are as follows:

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

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

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

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

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

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

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 μ^3 in controls, 408.61 μ^3 in treated animals; nucleus paraventricularis: 312.18 μ^3 in controls, 359.34 μ^3 in treated animals) and in the number of Gomori stained-positive glial cells per 0.076 mm^2 of the nucleus habenulae (controls: 5.75, treated animals: 6.88). The authors speculated that the increase in Gomori stained cells was a response designed to protect the brain from xenobiotic-induced toxicity. No speculation was offered to explain the cell volume changes, but it might be inferred that they may lead to further endocrine or reproductive effects.

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

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

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

Table III-14. NOEL and LOEL values in laboratory animal studies on the reproductive toxicity of carbaryl

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

[a] HDT, high dose tested; LDT, low dose tested
[b] This value is expressed as a dietary concentration because it was not possible to determine actual carbaryl intake in the pups.
[c] The internal dose levels in the gerbil study were calculated using published values for average body weight and food consumption in that species (Harkness and Wagner, 1983). Use of these default body weight and food consumption values was based on the unproven assumption that they were unaffected by the carbaryl intake.

G. DEVELOPMENTAL TOXICITY

1. Overview

Developmental toxicity studies in rats, rabbits and mice did not indicate specific problems 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-16.

2. Laboratory animal studies

a. Rats - gavage

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

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

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

b. Rabbits and mice - gavage

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

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

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

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

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

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

Rabbits, gavage. Diarrhea was observed at 200 mg/kg/day only (quantitative data not

provided). One death occurred among the controls and one among the 150 mg/kg/day group. These were not attributed to carbaryl exposure. Both controls and dosed animals lost weight over the gestation day 6-11 period, though the latter decrements were statistically greater than the controls (controls *vs.* 150 mg/kg/day, gestation days 6-11: -0.03±0.09 kg *vs.* -0.15±0.10* kg; controls *vs.* 200 mg/kg/day, -0.03±0.07 kg *vs.* -0.31±0.10* kg; *p<0.05).

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

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

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

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

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

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

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

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

c. Dogs - dietary

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

There were neither clinical signs nor discernable effects on maternal body weights during gestation. Dystocia, defined in this study as a “pattern” of difficult births [11], 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

[11] Symptoms included 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.

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, lessened 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 each 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-15 summarizes the maternal and pup data. Of the mated females, 9/12, 7/12,

9/12 and 9/12 became pregnant. One female in each carbaryl-exposed group died. The death reported at 2 mg/kg/day was an animal killed *in extremis* on day 48 due to poor health and convulsions. The mid and high dose deaths occurred at parturition; signs were not reported for those animals. The pattern of pregnancies and maternal deaths did not clearly implicate carbaryl. While the presence of convulsions in the low dose death might be construed as cholinergic, the absence of this sign at higher doses may indicate that it was not related to carbaryl exposure. The time of occurrence (day 48) for a sign that is more likely to be acute in nature, in addition to the absence of other cholinergic signs, also supported a non-carbaryl-dependent etiology. Effects of carbaryl on maternal body weight were not apparent.

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

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

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

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

The primary deficiencies in this study included a lack of dose analysis, no necropsies

performed on the mothers, mother's ages not reported, and the high dose may not have been sufficient. This study is considered to be supplemental.

Table III-15. Effect of subchronic exposure to dietary carbaryl on pregnant beagle dogs and their offspring; Immings *et al.* (1969)

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

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

Table III-16. NOEL and LOEL values for studies on the developmental toxicity of carbaryl

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

[a] HDT, high dose tested; LDT, low dose tested
[b] Dietary exposure probably continued through weaning, 8 weeks *post partum*, though the report was not explicit on this point.
[c] The designation "Acceptable" indicates that the study was successfully completed according to FIFRA guidelines. "Supplemental" indicates that the study was not conducted according to FIFRA guidelines; however, such studies were reviewed and considered to contribute to the general genotoxic picture of the chemical.

H. DEVELOPMENTAL NEUROTOXICITY

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

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

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

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

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

Results, F_0 animals (only females tested). There were neither treatment-related deaths nor signs detected in twice daily examinations. Reduced weight gains were noted at 10 mg/kg/day for the gd 6-9 period: 6.6, 7.7, 7.2 and 0.5** grams (**$p<0.01$) at ascending doses. FOB testing at 10 mg/kg/day revealed an increased incidence in dams with pinpoint pupils on all occasions during the dosing period ($p<0.05$-0.005), as well as in dams with slight tremors or slight ataxic gait on many occasions (Table III-17a). 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-17b). Suppression was noted on other measurement days as well, but didn’t achieve statistical significance. Brain ChE was statistically suppressed at the high dose on ppd 10, the only measurement day. The same trend was apparent for plasma ChE, though statistical significance was not indicated at any dose. Gross pathology did not reveal an

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

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

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

Conclusions. 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 result in a 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 a LED_{10} of 0.25 mg/kg (see section IV.1.a.). Consequently, both 1 mg/kg and 0.25 mg/kg might be used 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, as well as certain changes in brain morphometric measurements at that dose, may have resulted from carbaryl exposure.

This study was deemed acceptable according to FIFRA standards.

Table III-17a. Selected functional observational battery observations in F_0 females during the period of dosing with carbaryl (gd 6 - ppd 10), F_0 females (Robinson and Broxup, 1997)

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

Pinpoint pupils				
gd 6	0/23 (0)	1/26 (3.8)	0/26 (0)	9/23 (39.1)***
gd 9	0/26 (0)	0/26 (0)	0/26 (0)	4/26 (15.4)
gd 12	1/26 (3.8)	0/26 (0)	0/26 (0)	7/26 (26.9)*
gd 15	2/25 (8.0)	3/26 (11.5)	0/26 (0)	12/26 (46.2)***
gd 18	1/25 (4.0)	1/26 (3.8)	2/26 (7.7)	13/26 (50.0)***
gd 20	1/25 (4.0)	0/26 (0)	1/26 (3.8)	16/26 (61.5)***
ppd 4	0/21 (0)	1/23 (4.3)	0/24 (0)	6/24 (25.0)*
ppd 7	0/21 (0)	0/23 (0)	3/24 (12.5)	12/24 (50.0)***
ppd 11	0/21 (0)	1/23 (4.3)	0/24 (0)	0/24 (0)
ppd 13	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
ppd 21	0/21 (0)	0/23 (0)	0/24 (0)	0/24 (0)
Moderate dilation of pupils				
gd 6	2/23 (9)	7/26 (27)	5/26 (19)	1/23 (4)
gd 9	4/26 (15)	5/26 (19)	2/26 (8)	1/26 (4) [ɣ]
gd 12	2/26 (8)	0/26 (0) [ɣɣ]	2/26 (8)	0/26 (0) [ɣɣ]
gd 15	2/25 (8)	4/26 (15)	2/26 (8)	0/26 (0) [ɣɣ]
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) [ɣɣ]
ppd 4	0/21 (0)	1/23 (4)	1/24 (4)	0/24 (0)
ppd 7	3/21 (14)	6/21 (29)	4/24 (17)	5/24 (21)
ppd 11	2/21 (10)	4/23 (17)	4/24 (17)	8/24 (33)
ppd 13	1/21 (5)	2/23 (9)	2/24 (8)	2/24 (8)
ppd 21	1/21 (5)	3/23 (13)	3/24 (13)	1/24 (4)
Signs (per animal basis) [d]				
gd 6	6/23 (26.1)	7/26 (26.9)	7/26 (26.9)	16/23 (69.6)***
gd 9	7/26 (26.9)	2/26 (7.7)	10/26 (38.5)	12/26 (46.2)
gd 12	6/26 (23.1)	5/26 (19.2)	13/26 (50.0)*	14/26 (53.8)*
gd 15	8/25 (32.0)	12/26 (46.2)	14/26 (53.8)	12/26 (46.2)
gd 18	6/25 (24.0)	10/26 (38.5)	12/26 (46.2)	18/26 (69.2)**
gd 20	9/25 (36.0)	5/26 (19.2)	11/26 (42.3)	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.

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

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

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

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

[d] Signs (per animal basis) was an aggregate indicator of carbaryl effects noted in the FOB tests. They were enumerated by the risk assessor. Animals for which there were one or more signs were counted only once per test day. Only positive signs were considered (*i.e.*, the decline in incidence of moderate dilation of pupils was not included). Changes in defecation or urination were not included since they did not bear a clear relation to dose.

Table III-17b. RBC, plasma and brain cholinesterase activities in F_0 female CD rats during the period of dosing with carbaryl (gd 6 - ppd 10) (Robinson and Broxup, 1997)

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

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

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 Indredient 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_{50} and subchronic LOEL than carbaryl. In addition, as a decarbamylated degradate, 1-naphthol was unlikely to be an effective cholinesterase inhibtor, 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 ^{14}C at the 1-carbon was administered intraperitoneally to cats, pigs and rats at a dose of 25 mg/kg; at 24 hr, 91% of the radioactivity had been excreted in the urine of cats (98% sulfate conjugate,1.4% glucuronide conjugate), 81% in the pig (32% sulfate, 66% glucuronide) and 59% in the rat (53% sulfate, 47% glucuronide). Incubation of radiolabeled 1-naphthol with human blood for 24 hr resulted in the binding of 97.6% to plasma (92.8% of that in albumin, 3.6% in heavy lipoprotein and 3.6% in light lipoprotein fractions). In the same study, injection of mice with 1-naphthol, followed after 10 min by sacrifice and blood centrifuation showed 20-30% in the RBC fraction; the plasma fraction showed 43% associated with albumin and 43% with lipoproteins. A very limited study using three male volunteers determined that 1-naphthol contained in an ointment was rapidly absorbed. (CIR Expert Panel, 1989)

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

Poole and Buckley (1989), in the acute dosing section of a larger study (subchronic section below), treated two CD1 mice/sex/dose with 1-naphthol by gavage. The doses were 0-untreated

control, 0-vehicle control (vehicle: propane-1,2-diol : water, 1:1), 0.5, 1 or 2 g/kg 1-naphthol. Survivors were observed for up to 2 weeks post dose. Sacrifice was followed by *post mortem* exam, blood analysis (clinical chemistry and hematology), and fixation of major organs for histolopathologic analysis.

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

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

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

This study was considered to be supplemental.

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

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

Acute dermal toxicity. TSCA submission: "1-Naphthol (CAS # 90-15-3) was evaluated for acute dermal toxicity. The test substance was administered to 5 albino rabbits at a dosage of 10,000 mg/kg. No mortality and no signs of intoxication occurred. Dermal irritation consisted of moderate erythema and edema. Gross autopsy revealed no significant findings.
[Summary Results Concerning an Acute Oral LD50, Acute Eye Irritation, Primary Skin & Eye

Irritation Indexes & 28-Day Subacute Feeding Studies with Cover Sheet & Letter (Sanitized); 00/00/00; EPA No. 86-920000514S; Fiche No. OTS0533803]**UNREVIEWED**"

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

Subchronic oral toxicity. "1-Naphthol orally administered to rats (20 males and 20 females) for 12 weeks (5 times a week) showed that the dose of 20 mg/kg b.w./day (10 ml/kg) does not represent a toxic cumulative dose." (SCCNFP, 2001)

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

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

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

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

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

This study was considered to be supplemental.

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

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

Irritation (skin). "The compound was applied to intact and abraded skin of rabbit at doses of

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

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

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

TSCA submission: "1-Naphthol (CAS # 90-15-3) was evaluated for primary dermal irritation. The test substance was administered at a dosage of 500 mg to the intact and abraded skin of 6 albino rabbits. Moderate to severe erythema and edema was noted after 72 hours (irritation score of 7.09/8.00).
[Summary Results Concerning an Acute Oral LD_{50}, Acute Eye Irritation, Primary Skin & Eye Irritation Indexes & 28-Day Subacute Feeding Studies with Cover Sheet & Letter (Sanitized); 00/00/00; EPA No. 86-920000514S; Fiche No. OTS0533803]**UNREVIEWED**"

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

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

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

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

"Sensitization was induced in 20 guinea pigs by simultaneously intradermal injections in the shoulder region of 0.1 ml of Freund's Complete Adjuvant (FCA), 0.1 ml 1-naphthol (0.1% in water) and a 1:1 mixture of test compound and 0.05 ml Adjuvant at day 0. The test compound was dermally applied (0.1% in water) 7 days later, under occlusion, on the injection site for 48 hours. 14 days later the guinea pigs were challenged by dermal application on the flank with 0.1% and 0.05% of 1-naphthol (aqueous solutions), under occlusion for 24 hours. The results

evaluated after 24 and 48 hours of challenge showed that 1-naphthol was not a sensitiser in guinea pigs. Result: The sensitisation capacity was not properly assessed because the choice of concentration, for induction and challenge, may have been too low." (SCCNFP, 2001)

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

"25 female Sprague-Dawley Albino rats/group; Dosage 20, 40, 80 mg/kg bw. 1-Naphthol daily day 6 to 15 of gestation; Blank control (solvent); positive control 15 mg/kg/ vit. A; Acknowledged methodologies. Results: At any dose level no treatment related effects. No maternal nor embryonic or foetal signs attributable to the test substance. In conclusion no maternal or embryo-toxicity, no incidence of embryo-lethality or growth retarding effects; no teratogenicity up to the highest tested dose of 80 mg/kg." (SCCNFP, 2001)

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

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

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

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

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

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

- Rat bone marrow cells were negative for chromosome aberrations.

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

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

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

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

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

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

2. Methylamine

Methylamine is produced upon hydrolytic breakdown of carbaryl, which occurs under alkaline conditions. This compound is known for its irritant properties to eyes, nose and throat upon brief exposures to 20 - 100 ppm. Severe methylamine exposure may lead to pulmonary edema. The oral LD_{50} in rats is 100 - 200 mg/kg (Proctor *et al*, 1988). Shelby *et al.* (1987) demonstrated positivity in the L5178Y mutagenicity assay. Gavage exposure of mice to 122 mg/kg methylamine for 5 days produced a statistically significant 27% drop in white blood cell counts (Keil *et al.*, 1996).

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

The critical NOELs and LEDs identified in the following sections are contained in Table VII-1 at the end of this document.

1. Non-oncogenic effects

a. Acute oral toxicity

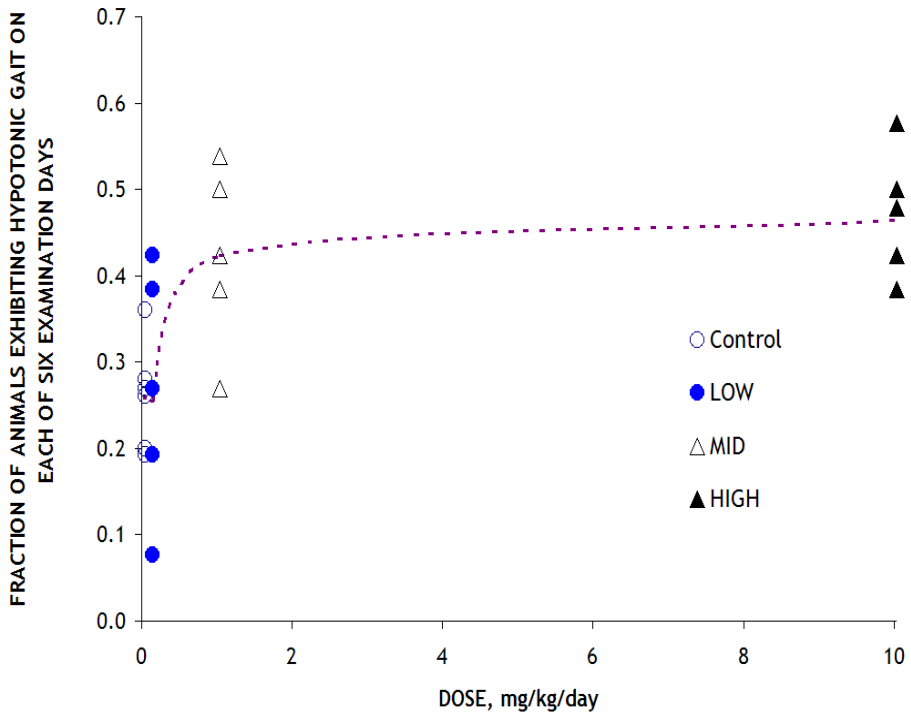
In terms of dose, the most sensitive acute (or short-term) toxicologic endpoints for carbaryl were identified in the gavage developmental neurotoxicity study of Robinson and Broxup (1997). Two plausible acute regulatory endpoint values, including a NOEL and an LED_{10}, were derived from this study.

(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 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% at 10 mg/kg ($p>0.05$) even at the first post dosing measurement on gd 6.

(2) A "lower bound on the effective dose at the 10% level" (LED_{10}) of **0.25 mg/kg** was obtained through benchmark dose (BMD) modeling of the slight hypotonic gait incidence data gathered during gestation. While these data were not as robust as the FOB and body weight data at 10 mg/kg, 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[12]; the dotted line is a representation of the average response at the doses tested.



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

but equivalent, acute scenarios [13]. 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_{10} 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_{10} was 0.25 mg/kg (ED_{10} = 0.47 mg/kg).

While both critical acute values---the NOEL of 1 mg/kg and the LED_{10} of 0.25 mg/kg---were plausible, this risk assessment used the NOEL of 1 mg/kg to calculate the acute MOEs for inhalation and oral exposure in the Risk Characterization section. This was done in recognition of the clearer experimental support for effects at 10 mg/kg. In no way, however, did this negate the possibility that the most sensitive neurobehavioral endpoints---hypotonic gait and tremors---were slightly induced even at 1 mg/kg. Indeed, use of the resultant 0.25 mg/kg LED_{10} would result in acute MOEs that are 4-fold less than those calculated for the relevant exposure scenarios. MOEs calculated using 1 mg/kg should be viewed with a high degree of seriousness since they could be underestimating the actual acute risk.

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

(1) The acute gavage study of Brooks and Broxup (1995b) demonstrated clear inhibition of all cholinesterases (including brain cholinesterase) at this dose. For example, at 0.5 hr post dose, brain cholinesterase activities at 10, 50 and 125 mg/kg were 46%, 23% and 18% of concurrent controls, respectively, while female activities were 54%, 24% and 22% of controls. Benchmark dose analysis using the Hill algorithm of the male data yielded LED_{10} (ED_{10}) values of 0.61 (0.95) mg/kg (Appendix II). 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

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

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; the data are also available in Moser *et al.*, 2010) demonstrated dose-dependent, statistically significant brain ChE inhibition in pnd11 rats at gavage doses as low as the lowest tested dose of 3 mg/kg. This resulted in a LED_{10} (ED_{10}) determination of 1.14 (1.46) mg/kg using benchmark dose methodology - essentially the same as the critical acute NOEL of 1 mg/kg and reasonably close to the LED_{10} of 0.25 mg/kg.

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

The critical chronic oral LED_{10} of 0.5 mg/kg, which was based on brain cholinesterase inhibition in dogs exposed by the dietary route (Hamada, 1987) also supported the critical acute NOEL and LED_{10} 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_{10} of 9.81 mg/m^3 by BMD analysis. This was based on inhibition of brain cholinesterase activity at a low dose of 10 mg/m^3 (1.2 mg/kg, calculated using the rat default breathing rate of 0.96 m^3/kg/day) in females after a single 3-hr exposure. The LED_{10} (ED_{10}) 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**
Slight hypotonic gait				
gd 6 [a]	6/23 (26.1) [b]	7/26 (26.9)	7/26 (26.9)	11/23 (47.8)
gd 9	7/26 (26.9)	2/26 (7.7)	10/26 (38.5)	11/26 (42.3)
gd 12	5/26 (19.2)	5/26 (19.2)	13/26 (50.0)*	11/26 (42.3)
gd 15	7/25 (28.0)	11/26 (42.3)	14/26 (53.8)	10/26 (38.5)
gd 18	5/25 (20.0)	10/26 (38.5)	11/26 (42.3)	15/26 (57.7)**
gd 20	9/25 (36.0)	5/26 (19.2)	11/26 (42.3)	13/26 (50.0)
Slight hypotonic gait (mean)	6.8/26 (26.2)	6.7/26 (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.
[a] Abbreviation: gd, gestation day.
[b] Numbers in parentheses are the incidence rates expressed in percentages.

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 activites were statistically suppressed in females at all doses for weeks 5, 13 and 26 (up to 23% at the low dose). Statistical significance in males occurred at the mid and high doses only.

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

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

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_{10} of 1.18 (1.70) mg/kg [14] 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_{10} 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_{10} 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_{10} based on the relatively low doses employed.

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

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[14] Internal dose = LED_{10} (or ED_{10}) x (rat breathing rate x 3 hr / 24 hr) = 9.8 (or 14.15) mg/m^3 x (0.96 m^3/kg/day x 3/24) = 1.18 (1.70) mg/kg

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

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

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

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

A developmental LOEL of 2 mg/kg/day was set in the study of Immings *et al.* (1969) based on a non-statistically significant increase in stillborn beagle dogs at that dose (Table III-15). 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-15 with its preceding discussion). Abnormalities---including umbilical hernia, cleft palate, fat-like mass in the heart, intussuception of the ileum into the colon, extravasation of blood in the myocardium and unilateral microphthalmia---were detected in the pups at the mid (5 mg/kg/day) and high doses (12.5 mg/kg/day). The co-incidence with the stillbirths and pup deaths was also attributed by the authors to maternal illness during the mating period. However, the proximity of the effective dose range in the two dog studies supported the possibility that there was an actual treatment effect.

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 chomosome aberration study in *Allium cepa* (onion tree), a system that was of questionable relevance to mammalian systems, all of the positive studies were performed *in vitro*. In general, positive *in vitro* assays may be less relevant to the whole organism than positive *in vivo* results, though they may provide mechanistic insights in some cases.

One study demonstrated that nitrosocarbaryl could be produced from carbaryl and nitrite under acidic *in vitro* conditions. A separate study showed that nitrosocarbaryl caused chromosomal aberrations in Chinese hamster fibroblasts. Finally, one study in V79 Chinese hamster fibroblasts showed that, like carbaryl, the carbaryl metabolite ∝-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-9c) (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-8c) (Hamada, 1993a). These were accompanied by possibly preneoplastic signs such as cellular hypertrophy or hyperplasia, squamous metaplasia, high mitotic index and/or atypia. In view of the positive genotoxicity tests (see previous section), it is premature to exclude genotoxicity as a possible driver in carbaryl-induced cancers in rodents, though direct evidence for genotoxically-driven tumors was lacking.

Mice. In view of the tendency of male mice to form hemangiosarcomas / hemangiomas at lower doses than were seen for the other tumors in this species, as well as the corroborating evidence from comparative benchmark dose analyses of all of the relevant mouse tumor data (see below; hemangiosarcoma / hemangioma, hepatocellular adenoma / carcinoma, kidney tubule cell adenoma / carcinoma), the human cancer risk was evaluated using the male mouse hemangiosarcoma / hemangioma data (Hamada, 1993b). Further support for use of this dataset came from knowledge that, while the high dose exceeded the maximum tolerated dose (MTD; this was based on early female deaths, body weight decrements, 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, 26th edition, p. 587). Hemangioma is defined as "a benign tumor made up of new-formed blood vessels" (Dorland's, p. 587). The more encompassing term angiosarcoma includes "all lesions labeled hemangiosarcoma, lymphangiosarcoma, and malignant hemangiosarcoma, since it remains uncertain whether these lesions are derived from blood vascular or lymphatic endothelium, or perhaps from either" (Fletcher and McKee, 1992). The latter definition is mentioned in this context because it emphasizes the unclear cellular origin of this type of tumor.

Incidences of hemangiomas and hemagiosarcomas were combined as recommended by the National Toxicology Program (McConnell *et al.,* 1986). This reflects the conviction that the underlying tumorigenic process was similar for the benign and malignant types. A dose-responsive increase was noted in males (dose response of "at risk" males: 2/66, 6/66, 10/69* and 10/68* at 0, 14.73, 145.99 and 1248.93 mg/kg/day; *p<0.05; Table III-9c). 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-9c)). A USEPA-sponsored reanalysis of the the pathology slides generated very similar results with respect to all tumors noted, both in the mouse and rat (USEPA, 2002b). However, small changes with respect to incidence of hemangiosarcomas alone in male mice, from 2/66, 5/66, 9/69* and 7/69 (calculated from the incidences of this lesion among interim, unscheduled and terminal sacrifices) to 1/66, 6/66*, 8/69* and 8/68*, further emphasized the likelihood that a tumorigenic response was present even at the low dose in the mouse study.

The mouse internal doses were converted to equivalent human doses and the human potency values calculated using the multistage-cancer model within the benchmark dose application software (USEPA version 1.10). 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±2.2 g. Accordingly, the dose adjustment factor was:

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

The mean male mouse internal doses of 0, 14.73, 145.99 and 1248.93 mg/kg/day were thus converted using this factor to equivalent human doses of 0, 2.25, 22.34 and 191.09 mg/kg/day, from which the potency values were obtained by benchmark dose analysis. 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 resultant human oncogenic potency (also referred to as the Multistage Cancer Slope Factor) was **9.72×10^{-3} mg/kg/day^{-1}** at the 95% upper bound (Appendix IV). This factor 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-8c), 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 [15] 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 [16]. 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-8a) suggested that the mid dose was at least close to an MTD, as stated above in the summary of the study (section III.D.2.). In addition, the mid dose female hepatocellular adenoma incidence rate was similar to that in mid dose males, where there was no evidence of a dose-response relation for this tumor type (1/66, 1/67, 3/69, 1/67 at ascending doses). This may reflect the fact that the 4.3% incidence rate at that dose did not exceed the published historical control range of 0-6.3% for this tumor in female Sprague-Dawley rats (CPRC, 1994, quoted in USEPA, 2002b). These considerations raised the possibility that the mid dose incidence in females was either unrelated to carbaryl exposure or occurred at a dose too high for consideration in a quantitative risk assessment.

One further point should be made with regard to the rat study. As noted above, the US EPA argued that the mid dose bladder preneoplasias at week 53 found in the reanalysis may have developed into full tumors at a higher (but presumably still sub-MTD) dose. Their contention that the mid dose was below the MTD supported the qualitative relevance of the study to cancer risk

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

[16] 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.

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. However, 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 contributed to the view that carbaryl was carcinogenic in at least two mammalian species.

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, 2014). Exposure estimates from that document are summarized below and in the ensuing tables.

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, 2014). 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) [a]			SADD (mg/kg/day) [b]			AADD (mg/kg/day) [c]			LADD (mg/kg/day) [d]		
	Dermal	Inhalation	Total	Dermal	Inhalation	Total	Dermal	Inhalation	Total	Dermal	Inhalation	Total
Handlers: aerial applications (DPR, 2014: Tables 22 and 23)												
Aerial (liquids)												
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
High-acre aerial (liquid)												
Mixer / loader	26.0	0.0189	26.0									
Applicator	0.223	0.00236	0.226									
Flagger	0.239	0.00175	0.241									
High-acre aerial (granule)												
Loader	0.527	0.0283	0.556									
Applicator	0.0731	0.116	0.189									
Flagger	0.00994	0.00151	0.0115									
Handlers: airblast and groundboom applications (DPR, 2014: Tables 24 and 25)												
Airblast												
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 [e]	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 [f]	4.01	0.0302	4.04	0.961	0.00722	0.968	0.160	0.00120	0.161	0.0854	0.000854	0.0860
Groundboom												
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
High-acre groundboom												
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

Handlers: hand-held applications (DPR, 2014: Tables 26 and 27)												
Right-of-way Mixer / loader Applicator	1.45 48.8	0.0105 0.0176	1.46 48.8									
Backpack sprayer Mixer / loader / applicator	5.34	0.00598	5.35	0.619	1.27×10^{-4}	0.619	0.361	7.38×10^{-5}	0.361	0.193	3.94×10^{-5}	0.193
High-pressure handwand Mixer / loader / applicator	41.3	1.26	42.6	0.191	5.80×10^{-3}	0.197	0.111	3.38×10^{-3}	0.114	0.0593	1.80×10^{-3}	0.0611
Low-pressure handwand Mixer / loader / applicator	0.260	0.00176	0.262	0.0113	1.64×10^{-4}	0.0115	0.00662	9.55×10^{-5}	0.00671	0.00353	5.09×10^{-5}	0.00358
Trigger spray applicator Mixer / loader / applicator	0.000939	7.59×10^{-6}	0.000946	0.0000921	2.16×10^{-6}	0.0000943	0.0000537	1.26×10^{-6}	0.0000550	0.0000286	6.73×10^{-7}	0.0000293
Hose-end sprayer Mixer / loader / applicator	0.580	0.000854	0.581	0.00694	3.46×10^{-5}	0.00697	0.00405	2.20×10^{-5}	0.00407	0.00216	1.08×10^{-5}	0.00217
Handlers: ground applications of carbaryl dust and granular products (DPR, 2014: Tables 28 and 29)												
Broadcast spreader Mixer / loader Applicator	0.0576 0.0125	0.0251 0.00167	0.0827 0.0141	0.0104 0.00224	0.00454 0.000299	0.0149 0.00254	0.00259 0.000560	0.00113 0.0000749	0.00372 0.000635	0.00138 0.000299	0.000605 0.0000399	0.00199 0.000339
High-acre broadcast spreader Mixer / loader Applicator	0.144 0.0312	0.0629 0.00417	0.207 0.0353	0.0143 0.00308	0.00624 0.000412	0.0205 0.00349	0.00356 0.000770	0.00156 0.000103	0.00512 0.000873	0.00190 0.000411	0.000832 0.0000549	0.00273 0.000466
Push-type spreader 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
Dust applicator 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 (2014). 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.

[a] 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%.

[b] 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 (2014)

[c] 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, 2014).

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

[e] 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.

[f] Open-cab airblast applications at rates less that 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) [a]	SADD (mg/kg/day) [b]	AADD (mg/kg/day) [c]	LADD (mg/kg/day) [d]
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 30 and 31 (DPR, 2014). 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.

[a] 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 μg/cm^2 at the expiration of the restricted entry or pre-harvest interval (DPR, 2014: Tables 16 and 30)
TC, transfer coefficient in cm^2/hr (DPR, 2014: Table 30)
ED, exposure duration in hr/day

[b] SADD [i.e., seasonal average daily dosage] = [DA x ltDFR x TC x ED] ÷ body weight (70 kg)
ltDRR, long-term dislodgeable foliar residue (DPR, 2014: Table 31)

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

[d] 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 negligable. 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) [a] Dermal	Inhalation	Total
Residential handlers (DPR, 2014: Table 32)			
Backpack mixer / loader / applicator	0.163	0.000182	0.163
Low pressure handwand mixer / loader / applicator	0.00792	0.0000535	0.00794
Trigger spray applicator	0.000939	0.00000759	0.000946
Hose-end mixer / loader / applicator	0.00707	0.0000104	0.00708
Dust loader / applicator	0.136	0.0101	0.146
Push-type spreader loader / applicator	0.0259	0.000188	0.026
Residential reentry onto carbaryl-treated turf (DPR, 2014: Table 33)			
Adults [b]	2.58		2.58
Toddlers [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 32 and text in DPR (2014). Only short-term uses were anticipated for residential handler scenarios; consequently, there were no SADD, AADD or LADD estimates.

[a] $STADD_{handler}$ [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%.

$STADD_{reentry}$ = (95th percentile exposure rate in µg/kg/hr) x (2 hr/day) x (70% dermal absorption)

[b] 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 (2014). 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 (2014). 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	0.00125 [a]
Object-to-mouth transfer	0.00162 [b]
Soil ingestion	0.000229 [c]

Note: Calculation of the values in this table are futher elucidated in DPR (2014), pp. 83-84.

[a] For hand-to-mouth transfer, STADD = [(TTR) x (HSA) x (events/hr) x (% transferable, hand to mouth) x (% transferable, turf to hand) x (2 hr/day)] ÷ 15 kg

TTR, turf transferable residue = 0.939 µg/cm^2
HSA, hand surface area contacting mouth = 20 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)
% transferable, percent of residues transferable from turf to hand = 5% (or 0.05)

[b] For object-to-mouth transfer, STADD = [(TTR) x (OSA) x (% transferable)] ÷ 15 kg

TTR, turf transferable residue = 0.939 µg/cm^2
OSA, object surface area = 25 cm^2
% transferable, percent of residues transferable from hand to mouth with each contact = 100% (or 1.0)

[c] For soil ingestion,
STADD = [(8.28 lb ai/acre) x (0.1 g/day) x (1 cm) x (0.67 cm^3/g) x (4.54×10^8 µg/lb) x (2.47×10^{-8} acre/cm^2)] ÷ 15 kg

8.28 lb ai/acre = peak turf application rate
0.1 g/day = amount of soil ingested / day
0.67 cm^3/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 (2014).

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

	STADD [a] mg/kg/day	SADD [b] mg/kg/day	AADD [c] mg/kg/day
Adult	0.000043	0.00000016	0.000000000044
Child (6 yr)	0.00011	0.00000070	0.00000000019

[a] Estimations of STADD, SADD and AADD were executed as described in DPR (2014), Table 34. 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, 2014).

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 IV-6.

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

	Absorbed dose
Bystander exposure, agricultural applications (DPR, 2014: Table 35)	
1-hr absorbed dose (heavy activity) **Infant** **Adult**	0.0110 mg/kg/hr 0.00198 mg/kg/hr
Short-term absorbed daily dosage (STADD) **Infant** **Adult**	0.0192 mg/kg/day 0.00910 mg/kg/day
Seasonal absorbed daily dosage (SADD) **Infant** **Adult**	0.00469 mg/kg/day 0.00223 mg/kg/day
Annual absorbed daily dosage (AADD) **Infant** **Adult**	0.000391 mg/kg/day 0.000186 mg/kg/day
Lifetime absorbed daily dosage (LADD) **Infant** **Adult**	n/a 0.000186 mg/kg/day
Bystander exposure, public pest control programs (DPR, 2014: Table 36)	
1-hr absorbed dose (heavy activity) **Infant** **Adult**	0.0030mg/kg/hr 0.00054 mg/kg/hr
Short-term absorbed daily dosage (STADD) **Infant** **Adult**	0.00015 mg/kg/day 0.000027 mg/kg/day

Note: Assumptions underlying the calculations in this table are found in DPR (2014), Tables 35 and 36.

7. Ambient exposure

Exposure to carbaryl in the air that is not associated with specific applications may occur, though as noted in DPR (2014, p. 88), “ambient air monitoring conducted in Fresno, Tulare and Kings countes by ARB [the California Air Resources Board] did not detect carbaryl. Exposures to carbaryl in the ambient air are anticipated to be equal to or less than bystander exposures to carbaryl, as the highest pesticide concentrations in air occur adjacent to an application. Bystander exposure estimates are thus health-protective estimates for airborne carbaryl exposures both adjacent to and away from applications.”

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. As all of the critical endpoints used in this report were derived from animal studies, the target MOE of 100 was considered adequate. It should be noted, however, that an additional uncertainty factor related to possible developmental or reproductive effects was not considered for this document, even though such sensitivities may exist (see section VI.C.)

As noted in the accompanying exposure assessment document (DPR, 2014) 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, and (3) air monitoring studies designed to estimate bystander and ambient exposures by the inhalation route. 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 Multistage Cancer Slope value---9.72×10^{-3} $mg/kg/day^{-1}$ 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}. The highest oncogenic risk was calculated for citrus pruners (re-entry), at 1.38×10^{-2}.

These tables also provide MOEs for aggregate exposures, which were calculated for non-oncogenic effecits using the "hazard index" approach and for oncogenic effects by adding the risks incurred through the dermal, inhalaltion and dietary pathways. The non-oncogenic aggregate risk calculations assumed acute and chronic dietary MOEs of 228 and 1973, respectively, for the working population [17]. The oncogenic aggregate risk calculations assumed a dietary aggregate risk of 3.68×10^{-6} established for the population of the Western USA (DPR, 2010).

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 TableIV-7a 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.

[17] 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, 2014). 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 [d]		
	Dermal [a]	Inhalation [b]	Aggregate [c]	Dermal [a]	Inhalation [b]	Aggregate [c]	Dermal [a]	Inhalation [b]	Aggregate [c]	Dermal	Inhalation	Aggregate
Handlers: aerial applications												
Aerial (liquids)												
Mixer / loader	0.23	23	0.23 (0.23)	11	552	11	45	2212	44 (43)	$1.62x10^{-3}$	$1.18x10^{-6}$	$1.62x10^{-3}$ ($1.62x10^{-3}$)
Applicator	29	182	25 (23)	1308	4425	1010	5224	17,730	4035 (1325)	$1.39x10^{-5}$	$1.46x10^{-7}$	$1.40x10^{-5}$ ($1.77x10^{-5}$)
High-acre aerial (liquid)												
Mixer / loader	0.54	53	0.53 (1)									
Applicator	63	424	55 (44)									
Flagger	59	571	53 (43)									
High-acre aerial(granule)												
Loader	27	35	15 (15)									
Applicator	192	9	8 (8)									
Flagger	1408	662	450 (151)									
Handlers: airblast and groundboom applications												
Airblast												
Mixer / loader	2	20	2 (2)	9	44	7	18	88	16 (15)	$4.05x10^{-3}$	$2.94x10^{-5}$	$4.08x10^{-3}$ ($4.08x10^{-3}$)
Applicator, citrus	24	82	18 (17)	105	182	67	211	365	134 (125)	$3.44x10^{-4}$	$7.12x10^{-6}$	$3.51x10^{-4}$ ($3.55x10^{-4}$)
Applicator, 5-7.5 lb	42	24	15 (14)	215	43	36	859	171	143 (133)	$8.43x10^{-5}$	$1.52x10^{-5}$	$9.95x10^{-5}$ ($1.03x10^{-4}$)
Applicator, <5 lb	3	33	3 (3)	16	69	13	88	417	73 (70)	$8.30x10^{-4}$	$8.30x10^{-6}$	$8.38x10^{-4}$ ($8.42x10^{-4}$)

Groundboom Mixer / loader Applicator	6 103	493 13,077	6 (6) 102 (71)	34 561.43	166 295.86	28 193.76	135 2284	663 1182	112 (106) 779 (558)	5.40×10^{-4} 3.18×10^{-5}	3.91×10^{-6} 2.20×10^{-6}	5.44×10^{-6} (5.48×10^{-4}) 3.40×10^{-5} (3.77×10^{-5})
High-acre groundboom Mixer / loader Applicator	2 41	24 43	2 (2) 21 (19)	24 415	120 215	20 141	98 1661	481 861	81 (78) 567 (440)	7.43×10^{-4} 4.37×10^{-5}	5.37×10^{-6} 3.01×10^{-6}	7.48×10^{-4} (7.52×10^{-4}) 4.67×10^{-5} (5.04×10^{-5})
Handlers: hand-held applications												
Right-of-way Mixer / loader Applicator	10 0.29	95 57	9 (9) 0.29 (0.29)									
Backpack sprayer Mixer / loader / applicator	3	167	3 (3)	23	3937	61	39	6775	39 (38)	1.88×10^{-3}	3.83×10^{-7}	1.88×10^{-3} (1.88×10^{-3})
High-pressure handwand Mixer / loader / applicator	0.34	0.79	0.24 (0.24)	73	86	40	126	148	68 (66)	5.76×10^{-4}	1.75×10^{-5}	5.94×10^{-4} (5.97×10^{-4})
Low-pressure handwand Mixer / loader / applicator	54	568	49 (41)	1239	3049	881	2258	5236	1578 (877)	3.43×10^{-5}	4.95×10^{-7}	3.48×10^{-5} (3.85×10^{-5})
Trigger spray applicator Mixer / loader / applicator	14,909	131,752	13,394 (224)	152,009	231,481	91,755	260,708	396,825	157,339(1949)	2.78×10^{-7}	6.54×10^{-9}	2.85×10^{-7} (3.96×10^{-6})
Hose-end sprayer Mixer / loader / applicator	24	1171	24 (21)	2017	14,451	1770	3457	22,727	3001 (1190)	2.10×10^{-5}	1.05×10^{-7}	2.11×10^{-5} (2.48×10^{-5})

Handlers: ground applications of carbaryl dust and granular products												
Broadcast spreader												
Mixer / loader	243	40	34 (30)	1346	110	102	5405	442	409 (338)	$1.34x10^{-5}$	$5.88x10^{-6}$	$1.93x10^{-5}$ ($2.30x10^{-5}$)
Applicator	1120	599	390 (144)	6250	1672	1319	25,000	6676	5269 (1435)	$2.91x10^{-6}$	$3.88x10^{-7}$	$3.30x10^{-6}$ ($6.98x10^{-6}$)
High-acre broadcast spreader												
Mixer / loader	97	16	14 (13)	979	80	74	3933	321	296 (258)	$1.85x10^{-5}$	$8.09x10^{-6}$	$2.66x10^{-5}$ ($3.03x10^{-5}$)
Applicator	449	240	156 (93)	4545	1214	956	18,182	4854	3831(1302)	$3.99x10^{-6}$	$5.34x10^{-7}$	$4.52x10^{-6}$ ($8.20x10^{-6}$)
Push-type spreader												
Loader / applicator	54	532	49 (40)	639	1992	484	1094	3401	828 (583)	$6.63x10^{-5}$	$7.60x10^{-7}$	$6.71x10^{-5}$ ($7.07x10^{-5}$)
Dust applicator												
Loader / applicator	103	99	50 (41)	553	307	197	946	526	338 (289)	$7.65x10^{-5}$	$4.93x10^{-6}$	$8.14x10^{-5}$ ($8.51x10^{-5}$)

Note: The exposure values appear above in Table IV-2a. 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.

[a] 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.

[b] Acute inhalation NOEL = 1 mg/kg (acute oral study of Robinson and Broxup, 1997). Subchronic and chronic inhalation LED_{10} = 0.5 mg/kg/day (chronic oral study of Hamada, 1987).

[c] 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 aggregate acute or chronic risk for dermal, inhalation *and* dietary exposure. The aggregate 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

aggregate 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.

[d] Oncogenic risk was calculated as the product of the potency value in $mg/kg/day^{-1}$ 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 and 100% oral absorption was assumed. Thus for aerial liquid exposure to mixer/loaders by the dermal route: (9.72×10^{-3} $mg/kg/day^{-1}$) (0.167 mg/kg/day) = 1.62×10^{-3}. Aggregate oncogenic risk was either the sum of the dermal + inhalation risks or the sum of the dermal + inhalation + dietary risks (the latter aggregate value appears in parentheses). The dietary risk term was 3.68×10^{-6} (Western region) (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[a]	MOE, seasonal[a]	MOE, annual[a]	Oncogenic risk[b]
Apple hand thinning	4 (4)	7	27 (27)	2.68×10^{-3} (2.68×10^{-3})
Asparagus hand harvesting	4 (4)			
Beans scouting	19 (18)			
Blackberry pruning	4 (4)			
Cabbage scouting	10 (10)			
Citrus pruning	2 (2)	3	5 (5)	1.38×10^{-2} (1.38×10^{-2})
Corn detasseling	3 (3)	5	61 (59)	1.18×10^{-3} (1.18×10^{-3})
Cucumber scouting	33 (29)	58,333	140,000 (1946)	5.15×10^{-3} (5.15×10^{-3})
Grape leaf pulling	8 (8)	44	263 (232)	2.76×10^{-4} (2.80×10^{-4})
Lettuce scouting	12 (11)	20	122 (115)	5.96×10^{-4} (6.00×10^{-4})
Olive pruning	73 (55)	202	24,221 (1824)	2.99×10^{-5} (3.36×10^{-5})
Ornamental plant hand harvesting	107 (73)	164	328 (281)	2.22×10^{-4} (2.26×10^{-4})
Potato scouting	14 (13)	105	252 (223)	2.88×10^{-4} (2.92×10^{-4})
Strawberry scouting	109 (74)	394	787 (562)	9.20×10^{-5} (9.57×10^{-5})
Tobacco hand harvesting	18 (17)			
Tomato staking / tying	39 (33)	158	631 (478)	1.15×10^{-4} (1.19×10^{-4})
Turf maintenance	5 (5)			

Note: The exposure values appear above in Table IV-2b. Aggregate 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 aggregate 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. The aggregate oncogenic risk was the sum of the dermal oncogenic risk and the dietary risk value of 3.68×10^{-6} for the Western USA (DPR, 2010). 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 following Table IV-7a).

[a] 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.

[b] Oncogenic risk was calculated as the product of the potency value in $mg/kg/day^{-1}$ 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 and 100% absorption was assumed. Thus for apple hand thinners: (9.72×10^{-3} $mg/kg/day^{-1}$) (0.276 mg/kg/day) = 2.68×10^{-3}.

3. Residential handler and residential reentry exposure risks

Short-term MOEs for residential handler and residential reentry scenarios appear in Table IV-8. One acute dermal scenario, backpack mixer / loader / applicator, exhibited an MOE of less than 100. One short-term inhalation scenario MOE, duster loader / applicator, was also just less than 100. Residential reentry onto carbaryl-treated turf showed dermal MOEs of less than 10 for both adults and toddlers. Aggregate MOEs for all scenarios were even lower.

Seasonal, annual and lifetime exposures were not anticipated for reentry scenarios.

Table IV-8. Residential handler and residential turf reentry risks from exposure to carbaryl by the dermal and inhalation routes - short-term margins of exposure

Exposure scenario	Short-term MOEs [a]		
	Dermal [b]	Inhalation [c]	Aggregate [d]
Residential handlers			
Backpack mixer / loader / applicator	86	5495	85 (62)
Low pressure handwand mixer / loader / applicator	1768	18,692	1615 (200)
Trigger spray applicator	14,909	131,752	13,394 (224)
Hose-end mixer / loader / applicator	1980	96,154	1940 (204)
Dust loader / applicator	103	99	50 (49)
Push-type spreader loader / applicator	541	5319	491 (393)
Residential reentry onto carbaryl-treated turf			
Adults [e]	5		5 (5)
Toddlers [f]	3		3 (3)

Note: Only short-term uses were anticipated for residential handler scenarios; consequently, there were no SADD, AADD or LADD estimates. MOEs were rounded to the closest integer values (see Note to Table IV-7a).

[a] MOE calculations utilized the exposure values listed in Table IV-3. The hazard index approach was used to establish the "total" (combined) toxicity.

[b] Because dermal absorption under all scenarios was 70% and the critical acute 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. Acute dermal NOEL = 20 mg/kg x 0.7 = 14 mg/kg (Austin, 2002a).

[c] Acute inhalation NOEL = 1 mg/kg (acute oral study of Robinson and Broxup, 1997).

[d] 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", Table IV-7a).

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 [a]
Hand-to-mouth transfer	800 (82)
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).

[a] Acute oral NOEL = 1 mg/kg (acute oral study of Robinson and Broxup, 1997). Aggregate MOEs were created by adding exposure from dietary sources using the hazard index approach as described in Table IV-7a 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, since 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 [a]	Seasonal MOE [b]	Annual MOE [b]
Adult	2.33×10^{4}	3.12×10^{6}	1.14×10^{10}
Child (6 yr)	9.09×10^{3}	7.14×10^{5}	2.63×10^{9}

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).

[a] Acute oral NOEL / LED_{10} = 1 mg/kg (Robinson and Broxup, 1997).

[b] Subchronic and chronic oral NOEL = 0.5 mg/kg/day (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 adult bystanders was 1.81×10^{-6} (or 5.49×10^{-6} if dietary exposure is added). Oncogenic risk to infants was not calculated because DPR assumes that a lifetime of exposure underlies cancer development. However, exposure during vulnerable prenatal, postnatal or juvenile periods---in the absence of, or in addition to, lifetime exposure---may increase cancer risk (OEHHA, 2009b). For carbaryl, this approach not only increases the oncogenic risk estimate for lifetime exposures from 1.81×10^{-6} to 3.06×10^{-6} (or from 5.49×10^{-6} to 6.78×10^{-6} if dietary exposure is included) but highlights the possibility that there may be significant risk resulting from early lifestage exposure in the absence of lifetime exposure. For example, the risk from exposure *only* during the third trimester through age 2-year period was 0.58×10^{-6}. These calculations appear in the footnote below [18].

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[18] If, for example, default ASFs (age sensitivity factors) of 10 (third trimester to age 2) and 3 (ages 2-16) are applied, the following oncogenic risk estimation is possible (OEHHA, 2009b):

	ASF	Duration	Risk
risk, third trimester to age 2	10	2.25/70 yr	0.58×10^{-6}
risk, age 2 to age 16	3	14/70 yr	1.09×10^{-6}
risk, age 16 to age 70	1	54/70 yr	1.40×10^{-6}
70-yr lifetime risk			3.06×10^{-6}
70-yr lifetime risk + dietary			6.78×10^{-6}

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

	MOE
Bystander exposure, agricultural applications	
1-hr risk (heavy activity) [a] **Infant** **Adult**	 91 (46) 505 (157)
Short-term risk [a] **Infant** **Adult**	 52 (33) 110 (74)
Seasonal risk [b] **Infant** **Adult**	 107 224
Annual risk [b] **Infant** **Adult**	 1279 (86) 2688 (210)
Lifetime oncogenic risk [c] **Infant** **Adult**	 n/a [d] 1.81×10^{-6} (5.49×10^{-6})
Bystander exposure, public pest control programs	
1-hr risk (heavy activity) [a] **Infant** **Adult**	 333 (72) 1852 (203)
Short-term risk [a] **Infant** **Adult**	 6667 (91) 37,037 (227)

Note: The exposure assumptions underlying the calculations in this table are found in DPR (2014), Tables 36 and 37, and in the accompanying text. Aggregate MOEs were calculated by adding exposure from dietary sources using the hazard index approach as described in Table IV-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). The aggregate oncogenic risk to bystanders of agricultural applications was calculated by adding the dietary risk value of 3.68×10^{-6} to the value of 1.81×10^{-6} due to inhalation exposure.

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

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

[c] Oncogenic risk was calculated as the product of the potency value, 9.72×10^{-3} 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.

[d] See text and footnote 18 for a discussion of possible enhanced oncogenic risk during vulnerable developmental periods

7. Ambient exposure risks

No independent ambient exposure estimates were made by DPR (2014). 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 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_{10} (ED_{10}) of 1.1 (1.5) mg/kg based on brain cholinesterase inhibition (as well as a second LED_{10} (ED_{10}) 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_{10} (ED_{10}) of 1.18 (1.70) mg/kg based on brain cholinesterase inhibition.

Both LED_{10}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 [19]. MOEs calculated using the 1 mg/kg NOEL may thus underestimate the actual risk by a factor of 4.

However, there were also uncertainties surrounding the 0.25 mg/kg LED_{10} 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. Close examination of the incidence rates reveals a lack of consistent dose responsiveness throughout the dose range at most time points. Even so, the mean dose-response curve in Table IV-1 and Figure 2 appears to support an effect at 1 mg/kg.

2. An effect of dosing on slight hypotonic gait may not have appeared until gd 9 (*i.e.*, after four applications) or gd 12 (after seven applications), 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 it is 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-17a 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 be sufficient for a notification or be classified as moderate in the eyes of another evaluator.

4. The most scientifically credible route to establishing a critical acute value using the incidence data for slight hypotonic gait was to model those data using BMD modeling. 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.

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[19] This was explicit in the dietary assessment, where the LED_{10} 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 with 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.

However, there were uncertainties inherent in the BMD approach. First, there was uncertainty associated with the chosen benchmark response level of 10%, since it was not known if slight hypotonic gait comprised a centrally or peripherally-based response. If centrally-based, for example, the risk represented by slight hypotonic gait might be better characterized by a benchmark response level of 5% rather than 10% (which is associated with milder effects). Second, the decision to delete the top dose, which was made in order to generate a curve of appropriate fit and an LED_{10} value of lower than 1 mg/kg (a dose that was plausibly and effect level), added uncertainty since it ignored actual data gathered in the experiment at the high dose. Third, the choice of the probit function over other algorithms added uncertainty because each algorithm generated different LED_{10} and ED_{10} values. And fourth, the decision to model the data using normalized mean incidence rates, which was a consequence of considering all of the FOB tests to be acute in nature, added uncertainty because it implied that data from single test days represented fluctuations around a mean. While this was considered the more likely scenario, it remained possible that it under- or overestimated 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_{10} of 0.5 mg/kg/day was based on inhibition of brain ChE activity after 1 year of exposure in dogs at a low dose of 3.4 mg/kg/day (actually, 3.7 mg/kg/day in females and 3.4 mg/kg/day in males (Hamada, 1987)). The absence of clinical signs and histopathology throughout the study, even at the high dose of 34 mg/kg/day, leaves open the possibility that the LED_{10} value was too low, since 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.

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_{10} (ED_{10}) 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_{10} 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 $<20x10^6$/ml) and teratospermia (>60% abnormal sperm forms) in that population.

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

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

The epidemiologic studies did not, however, make unambiguous associations between exposure and effect. The extent of carbaryl exposure, both with regard to time span and dose, was ill-defined and did not preclude 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 also 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 preclude 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. 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 precise nature of the 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_0 or F_1 reproductive indices, parental epididymal sperm counts, sperm motility / morphology, homogenization-resistant spermatid head counts, daily sperm production or efficiency of daily sperm production (Tyl *et al.,* 2001). There were, however, deleterious effects on pup body weights and survival, as well as delays in developmental parameters. A dietary study in gerbils 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 (Rybokova, 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 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 occured over the entire 2-month dog gestation period in the Smalley study and continued through the pre-weaning period in the Immings study. As such, it was difficult to determine if the effects were acute or subchronic in nature.

4. Khera (1976) noted that, unlike other mammals, the dog sheds immature diploid ova, which then undergo a period of maturation and reduction to haploidy before being receptive to sperm. This could generate an altered reproductive sensitivity to xenobiotics in the dog, decreasing its relevancy as a model for potential effects in the human. However, there is no evidence at this time to support such a claim regarding carbaryl 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.

6. With respect to the study of Immings *et al.* (1969) in particular, there was concern that many of the affected pups were conceived during a period of maternal illness.

Even with 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 ≤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 a likely response to carbaryl exposure. 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.

In conclusion, the studies reviewed for this document suggest that carbaryl has developmental and/or reproductive effects in humans and laboratory animals. Among laboratory animals, dogs appear to be the most sensitive species. In view of the unclear dose-response relation in the study of Immings *et al.* (1969)---which was also true for Smalley *et al.*, (1968)---it would be difficult to extrapolate the LOEL of 2 mg/kg/day to an estimated NOEL. Nonetheless, it is a reasonable assumption that such a NOEL would be less than the critical acute NOEL of 1 mg/kg and perhaps less than the subchronic / chronic LED_{10} of 0.5 mg/kg/day.

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 ∝-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 that 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.

It is important to note that the estimations of oncogenic potency for various exposure categories (mostly occupational, but also bystander) do not take into account the possibility that early lifestages may be more oncogenically vulnerable than mature stages. In a lengthy discussion of this issue, OEHHA (2009b) concluded that, in the absence of specific data, it may be appropriate to apply default age sensitivity factors (ASFs) to arrive at more refined oncogenicity estimates. While such considerations may not apply to occupational exposure scenarios, we calculated oncogenic risk to bystanders in footnote 18 using the default early lifestage ASFs developed in that document. Thus the oncogenic risk estimate for adult bystanders rose from 1.81×10^{-6} to 3.06×10^{-6} (or from 5.49×10^{-6} to 6.78×10^{-6} if dietary exposure is included) when possible early lifestage vulnerability was taken into account. Early lifestage exposure in the absence of lifetime exposure may also be associated with significant oncogenic risk. Even so, we are not aware that studies designed to evaluate lifestage vulnerability exist for carbaryl.

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 secion 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, 2014).

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_{10} 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_{10}. 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 (2014). Using the health protective standard of one extra case in a population of 10^6 individuals, carbaryl presents a greater than negligable risk (sometimes considerably greater) under many exposure scenarios.

D. CRITICAL TOXICITY ENDPOINTS---USEPA *vs.* DPR

USEPA outlined its 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_{10} value derived from brain cholinesterase inhibition data in postnatal day 11 rats (Moser, 2007). The USEPA PoD was essentially equivalent to DPR's critical acute NOEL of 1 mg/kg, 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_{10} of 0.5 mg/kg/day (ED_{10} = 1.7 mg/kg/day) was derived using benchmark dose methodology applied to cholinesterase inhibition data in the 1-year dog study of Hamada (1987).

4. Acute, subchronic and chronic dermal toxicity

USEPA assigned an LED_{10} 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_{10} 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_{10} 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 11.1 (USEPA: 8.75×10^{-4} mg/kg/day^{-1}; DPR: 9.72×10^{-3} mg/kg/day^{-1}). 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 this risk assessment, we examined 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. We viewed reproductive toxicity mainly through the lens of epidemiologic studies, which indicated potential reproductive problems in males. We 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
Acute oral toxicity	**Moser, 2007** **Acute oral toxicity - rat** LOEL value not determined (brain ChEI) LED_{10} = 1.1 mg/kg	**Robinson & Broxup, 1997** **Developmental ntx - rat** LOEL = 10 mg/kg (cholinergic signs) NOEL = 1 mg/kg
Acute, subchronic and chronic dermal toxicity	**Austin, 2002a** **4-wk dermal toxicity - rat** LOEL = 50 mg/kg/day (brain ChEI) LED_{10} = 30 mg/kg/day	**Austin, 2002a** **4-wk dermal toxicity - rat** LOEL = 50 mg/kg/day (brain ChEI) NOEL = 20 mg/kg/day
Acute inhalation toxicity	**Moser, 2007** **Acute oral toxicity - rat** LOEL value not determined (brain ChEI) LED_{10} = 1.1 mg/kg	**Robinson & Broxup, 1997** **Developmental ntx - rat** LOEL = 10 mg/kg (cholinergic signs) NOEL = 1 mg/kg
Subchronic and chronic inhalation toxicity	**Moser, 2007** **Acute oral toxicity - rat** LOEL value not determined (brain ChEI) LED_{10} = 1.1 mg/kg	**Hamada, 1987** **1-year chronic - dog** LOEL = 3.1 mg/kg/day (brain / RBC / plasma ChEI) LED_{10} = 0.5 mg/kg/day
Subchronic oral toxicity	n/a (USEPA does not discuss intermediate / seasonal oral exposure scenarios for carbaryl)	**Hamada, 1987** **1-year chronic - dog** LOEL = 3.1 mg/kg/day (brain / RBC / plasma ChEI) LED_{10} = 0.5 mg/kg/day
Chronic toxicity (oral, dermal & inhalation)	n/a (USEPA does not consider carbaryl to pose a chronic toxicity risk)	**Hamada, 1987** **1-year chronic - dog** LOEL = 3.1 mg/kg/day (brain / RBC / plasma ChEI) LED_{10} = 0.5 mg/kg/day
Oncogenicity	**Hamada, 1993b** **2-year chronic / onco - mouse** Dose-dependent hemangiosarcomas 95% UB potency = 8.75×10^{-4}	**Hamada, 1993b** **2-year chronic / onco - mouse** Dose-dependent hemangiosarcomas & hemangiomas 95% UB potency = 9.72×10^{-3}

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” (NRC, 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, 2014). 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 IV-11). 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 establisment 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 asociated 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 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_2 mortality, pnd 0-4, at 300 (5-6 mg/kg/day) and 1500 ppm (Tyl *et al.,* 2001). However, the relevance of the dog system to humans has been questioned in regard to the fetal and developmental effects (see discussions in sections III.G.2.c., IV.A.1.a, V.A.1.a, V.A.1.c., V.A.1.d. and V.D.). Also, 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.

Partly 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.

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} = critical oral NOEL ÷ 100

❖ To calculate the acute oral reference dose #1 ($RfD_{acute\#1}$):
Critical acute oral NOEL = 1 mg/kg:
$RfD_{acute\#1}$ = 1 mg/kg ÷ 100 = **0.01 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 mg/kg ÷ 100 = **0.0025 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 mg/kg/day ÷ 100 = **0.005 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} = (critical dermal NOEL ÷ 100) x 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 mg/kg/day ÷ 100) x 5/7 = **0.14 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_{10} 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_{10} to an air concentration using the default human breathing rate appropriate to the exposure time (1, 8 or 24 hours) [20], 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:

$RfC_{1\text{-hr}}$ = (1 mg/kg ÷ 0.25 m^3/kg/hr) ÷ 100 = **0.04 mg/m^3**

$RfC_{8\text{-hr}}$ = (1 mg/kg ÷ 0.20 m^3/kg/8-hr) ÷ 100 = **0.05 mg/m^3**

$RfC_{24\text{-hr}}$ = (1 mg/kg ÷ 0.59 m^3/kg/24-hr) ÷ 100 = **0.02 mg/m^3**

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

$RfC_{1\text{-hr}}$ = (1 mg/kg ÷ 0.045 m^3/kg/hr) ÷ 100 = **0.22 mg/m^3**

$RfC_{8\text{-hr}}$ = (1 mg/kg ÷ 0.093 m^3/kg/8-hr) ÷ 100 = **0.11 mg/m^3**

$RfC_{24\text{-hr}}$ = (1 mg/kg ÷ 0.28 m^3/kg/24-hr) ÷ 100 = **0.04 mg/m^3**

[20] 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^3/kg/day; active human infant (1-hr exposure time): 0.25 m^3/kg/hr; resting human adult (8- and 24-hr exposure times): 0.28 m^3/kg/day; active human adult (1-hr exposure time): 0.045 m^3/kg/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.

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

❖ For infants (considered to represent all children):
$RfC_{s/a}$ = (0.5 mg/kg/day ÷ 0.59 m^3/kg/day) ÷ 100 = **0.008 mg/m^3/day**

❖ For adults:
$RfC_{s/a}$ = (0.5 mg/kg/day ÷ 0.28 m^3/kg/day) ÷ 100 = **0.02 mg/m^3/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
Acute oral #1 **Rat gavage dvpmt. ntx. study, gd 6 - ppd 10 (Robinson & Broxup, 1997)**	cholinergic signs, brain ChEI and body weight gain decrements	**LOEL** 10 mg/kg **NOEL** 1 mg/kg	**$RfD_{acute\#1}$** 0.01 mg/kg
Acute oral #2 **Rat gavage dvpmt. ntx. study, gd 6 - ppd 10 (Robinson & Broxup, 1997)**	slight hypotonic gait	**LOEL** not determined [a] **LED_{10}** 0.25 mg/kg	**$RfD_{acute\#2}$** 0.0025 mg/kg
Seasonal-annual oral **Dog 1-yr dietary study (Hamada, 1987)**	brain ChEI	**LOEL** 3.4 mg/kg/day **LED_{10}** 0.5 mg/kg/day	**$RfD_{s/a}$** 0.005 mg/kg/day
Dermal **Rabbit 4-wk dermal study (Austin, 2002a)**	brain ChEI	**LOEL** 50 mg/kg **NOEL** 20 mg/kg [c]	**$RfD_{a/s}$** 0.14 mg/kg
Acute inhalation **Rat gavage dvpmt. ntx. study, gd 6 - ppd 10 (Robinson & Broxup, 1997)**	cholinergic signs, brain ChEI and body weight gain decrements	**LOEL** 10 mg/kg **NOEL** 1 mg/kg	***Infants*:** **$RfC_{1\text{-}hr}$** 0.04 mg/m^3 **$RfC_{8\text{-}hr}$** 0.05 mg/m^3 **$RfC_{24\text{-}hr}$** 0.02 mg/m^3 ***Adults*:** **$RfC_{1\text{-}hr}$** 0.22 mg/m^3 **$RfC_{8\text{-}hr}$** 0.11 mg/m^3 **$RfC_{24\text{-}hr}$** 0.04 mg/m^3
Seasonal-annual inhalation **Dog 1-yr dietary study (Hamada, 1987)**	brain ChEI	**LOEL** 3.4 mg/kg/day **LED_{10}** 0.5 mg/kg/day	**$RfD_{s/a}$ - infants** 0.008 mg/m^3 **$RfD_{s/a}$ - adults** 0.02 mg/m^3

[a] 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.
[b] PE, point estimate; MC, Monte Carlo estimate. The dietary subpopulations examined are those covered in the DEEM-FCID® dietary analysis. The dietary exposure values were taken from Tables IV-3 and IV-4. Two subpopulations - females 13+ pregnant / not nursing and females 13+ nursing - were excluded from this comparison due to an insufficient number of available user days for analysis.
[c] 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.

VIII. 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 (an additional uncertainty factor related to possible developmental or reproductive effects was not considered for this document, though such sensitivities may exist). 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. Actual short-term inhalation and oral risks calculated using the critical acute NOEL of 1 mg/kg (as with the MOEs above) may be underestimated since a more health-conservative acute LED_{10} of 0.25 mg/kg was available. Oncogenic risk exceeded the negligible risk standard of 10^{-6} among occupational handler and reentry workers, often by orders of magnitude, approaching or exceeding 10^{-2} in some cases. Oncogenic risk for adult bystanders of agricultural applications also exceeded 10^{-6} (*i.e.*, 1.81×10^{-6}).

Risk mitigation measures should be considered for carbaryl.

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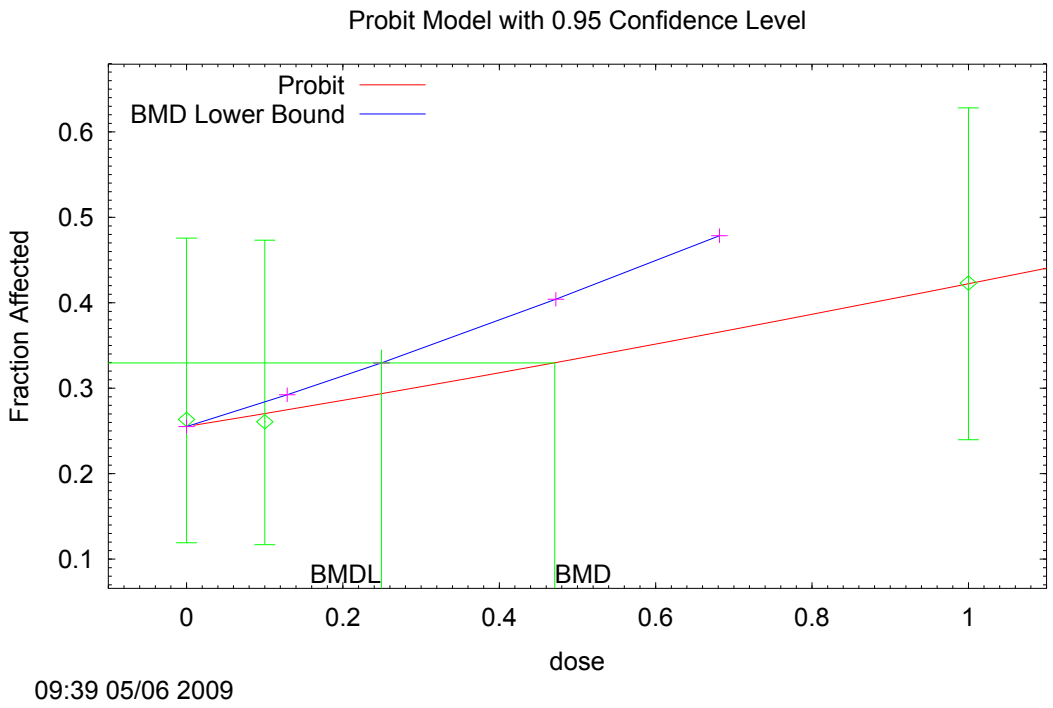
Appendix I. Benchmark dose extrapolation for induction of slight hypotonic gait in pregnant Sprague-Dawley rats (Robinson and Broxup, 1997)



Robinson and Broxup (1997) rat acute neurotoxicity study with carbaryl
Slight hypotonic gait data in females (top dose deleted)
Probit model; slope parameter not restricted
Risk type: "Extra risk"

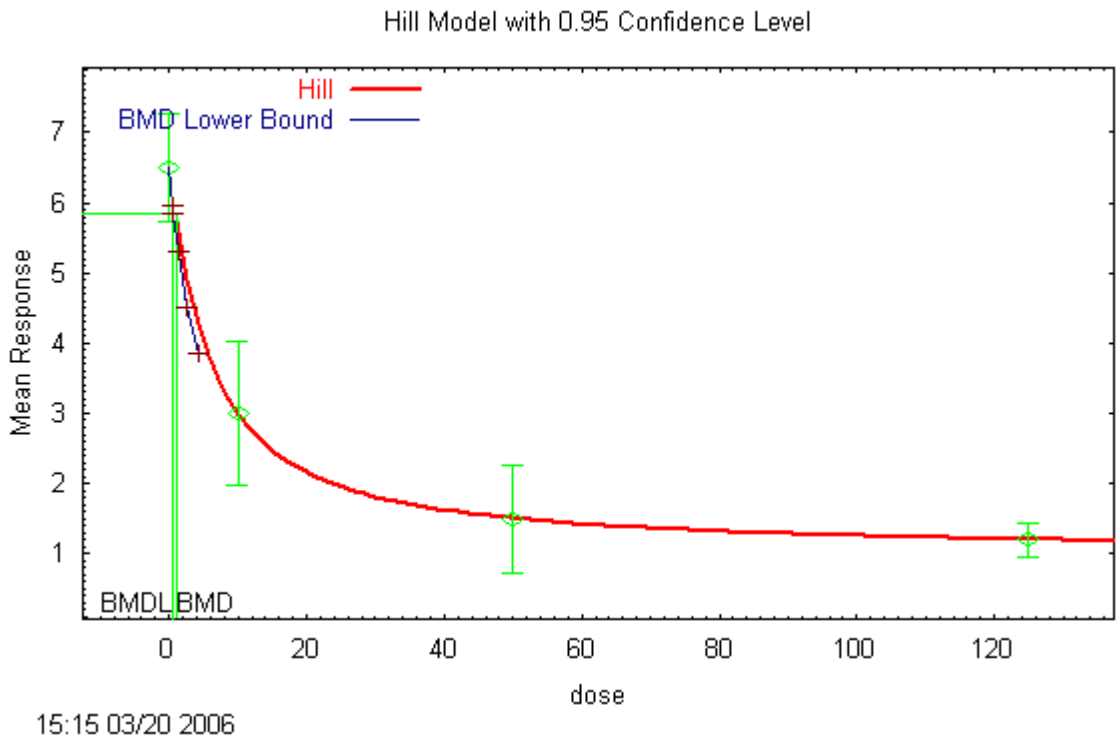
10% benchmark response:

```
 ==================================================================
         Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
         Input Data File:
C:\BMDS\DATA\CARBARYL_HYPOTONIC_GAIT_NORMALIZED_MEAN_GD_6_TO_20.(d)
         Gnuplot Plotting File:
C:\BMDS\DATA\CARBARYL_HYPOTONIC_GAIT_NORMALIZED_MEAN_GD_6_TO_20.plt
                                                Wed May 06 09:39:08 2009
 ==================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = COLUMN3
   Independent variable = COLUMN1
   Slope parameter is not restricted
```

```
   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial (and Specified) Parameter Values
                     background =            0   Specified
                      intercept =    -0.648276
                          slope =     0.454457


           Asymptotic Correlation Matrix of Parameter Estimates

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

              intercept        slope

 intercept            1        -0.65

     slope        -0.65            1


                          Parameter Estimates

       Variable           Estimate             Std. Err.
      intercept           -0.658436            0.198162
          slope            0.461902            0.326088


                        Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -47.3395
   Fitted model        -47.3503     0.0215168      1          0.8834
  Reduced model        -48.3536       2.02813      2          0.3627

           AIC:         98.7005


                     Goodness  of  Fit

                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     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 ChE activity in male rats at 0.5 hr post dose (Brooks and Broxup, 1995b)



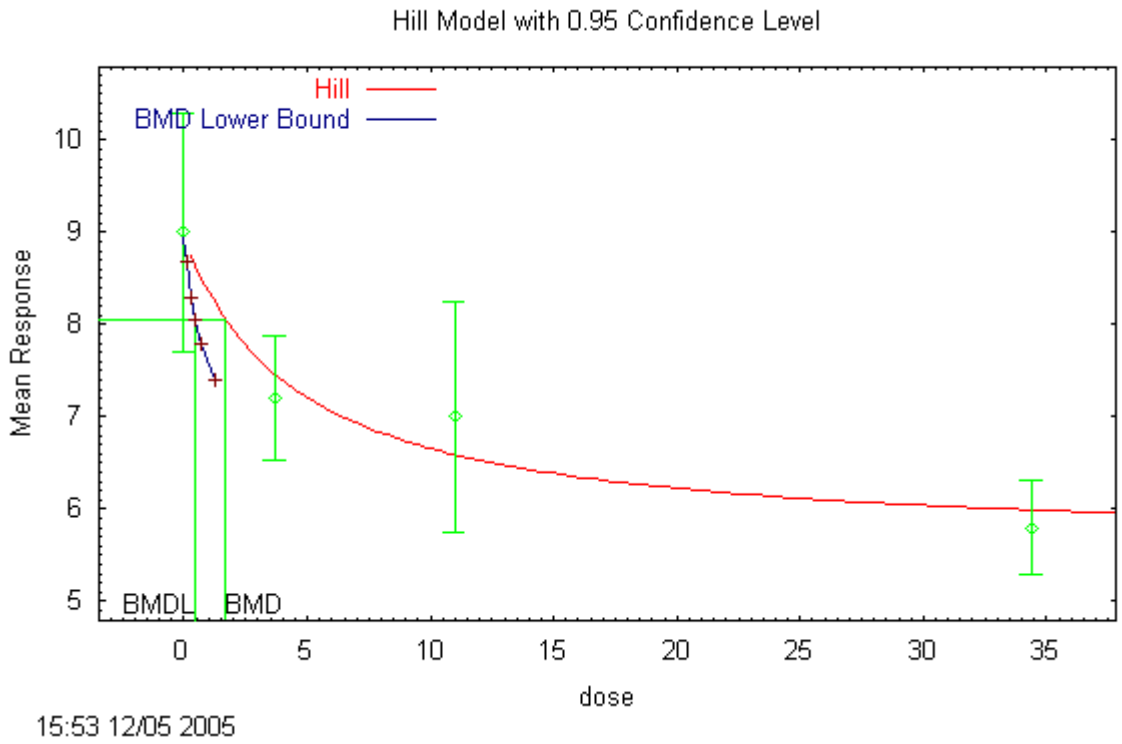
Brooks & Broxup (1995b) – male rats
Brain ChE activity, 0.5 hr
10% ED / LED
Relative risk

```
=====================================================================
         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 Mar 20 15:15:19 2006
 ====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = COLUMN1
   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 =    0.0627702
                          rho =            0   Specified
                    intercept =          6.5
                            v =         -5.3
                            n =      1.94035
                            k =      7.57143


           Asymptotic Correlation Matrix of Parameter Estimates

                  alpha          rho    intercept            v            n
k

     alpha            1            0            0            0            0
0

       rho            0            1            0            0            0
0

 intercept            0            0            1            0            0
0

         v            0            0            0            1            0
0

         n            0            0            0            0            1
0

         k            0            0            0            0            0
1


                         Parameter Estimates

       Variable           Estimate             Std. Err.
          alpha           0.0617167                   1
            rho                   0                   1
      intercept                 6.5                   1
              v            -5.49202                   1
              n             1.09048                   1
              k             5.96396                   1


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean    Obs Std Dev   Est Mean   Est Std Dev   Chi^2 Res.
------     ---   --------    -----------   --------   -----------   ----------

    0     3        6.5         0.31          6.5        0.248      8.81e-007
   10     3          3         0.41            3        0.248      1.49e-006
   50     3        1.5         0.31          1.5        0.248     -1.04e-006
  125     3        1.2          0.1          1.2        0.248     -1.57e-006


 Model Descriptions for likelihoods calculated
```

```
 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


Degrees of freedom for Test A1 vs fitted <= 0

                       Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
             A1           10.711208       5     -11.422415
             A2           13.042438       8     -10.084876
           fitted         10.711207       5     -11.422415
              R          -15.538817       2      35.077633

 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              57.1625          6          <.0001
   Test 2              4.66246          3          0.1982
   Test 3         1.38024e-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 .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 =      0.945771
            BMDL =      0.605523
```

Appendix III. Benchmark dose extrapolation for brain cholinesterase inhibition in female dogs after 52 weeks of exposure to dietary carbaryl (Hamada, 1987)



Hamada (1987) dog 1-yr dietary study with carbaryl
Brain cholinesterase data in females
Hill model, n>1
Risk type: "Relative risk"

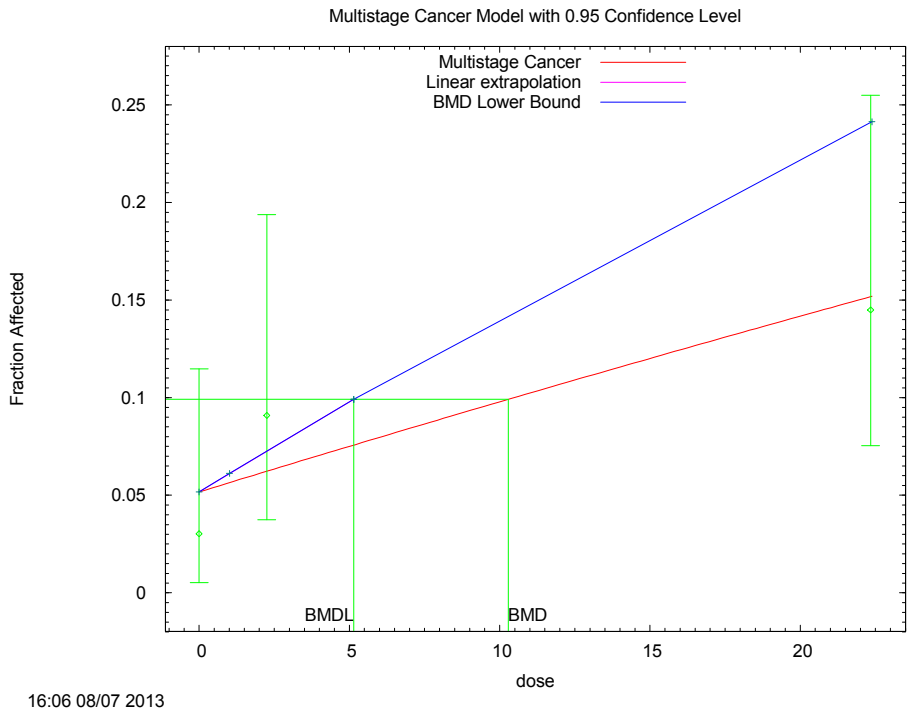
10% benchmark response:

```
=====================================================================
         Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
         Input Data File: D:\BMDS\UNSAVED1.(d)
         Gnuplot Plotting File:  D:\BMDS\UNSAVED1.plt
                                                Mon Dec 05 15:53:22 2005
 ====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
```

```
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                         alpha =     0.670558
                           rho =            0   Specified
                     intercept =            9
                             v =         -3.2
                             n =       1.2784
                             k =      3.28889


          Asymptotic Correlation Matrix of Parameter Estimates

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

                 alpha          rho    intercept            v            k

     alpha           1            0            0            0            0

       rho           0            1            0            0            0

 intercept           0            0            1            0            0

         v           0            0            0            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:        Yij = Mu(i) + e(ij)
```

```
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


Degrees of freedom for Test A1 vs fitted <= 0

                       Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
             A1           -8.444034       5      26.888069
             A2           -5.016409       8      26.032817
           fitted         -9.509932       4      27.019865
              R          -21.130889       2      46.261778

 Test 1:  Does response and/or variances differ among dose levels
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous (A1 vs A2)
 Test 3:  Does the Model for the Mean Fit (A1 vs. fitted)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df     p-value

   Test 1               32.229          6          <.0001
   Test 2              6.85525          3         0.07666
   Test 3               2.1318          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05.  A homogeneous variance
model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0.  The Chi-Square
     test for fit is not valid

 Benchmark Dose Computation
Specified effect =           0.1

Risk Type        =     Relative risk

Confidence level =          0.95

             BMD =       1.69014

            BMDL =       0.46739
```

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

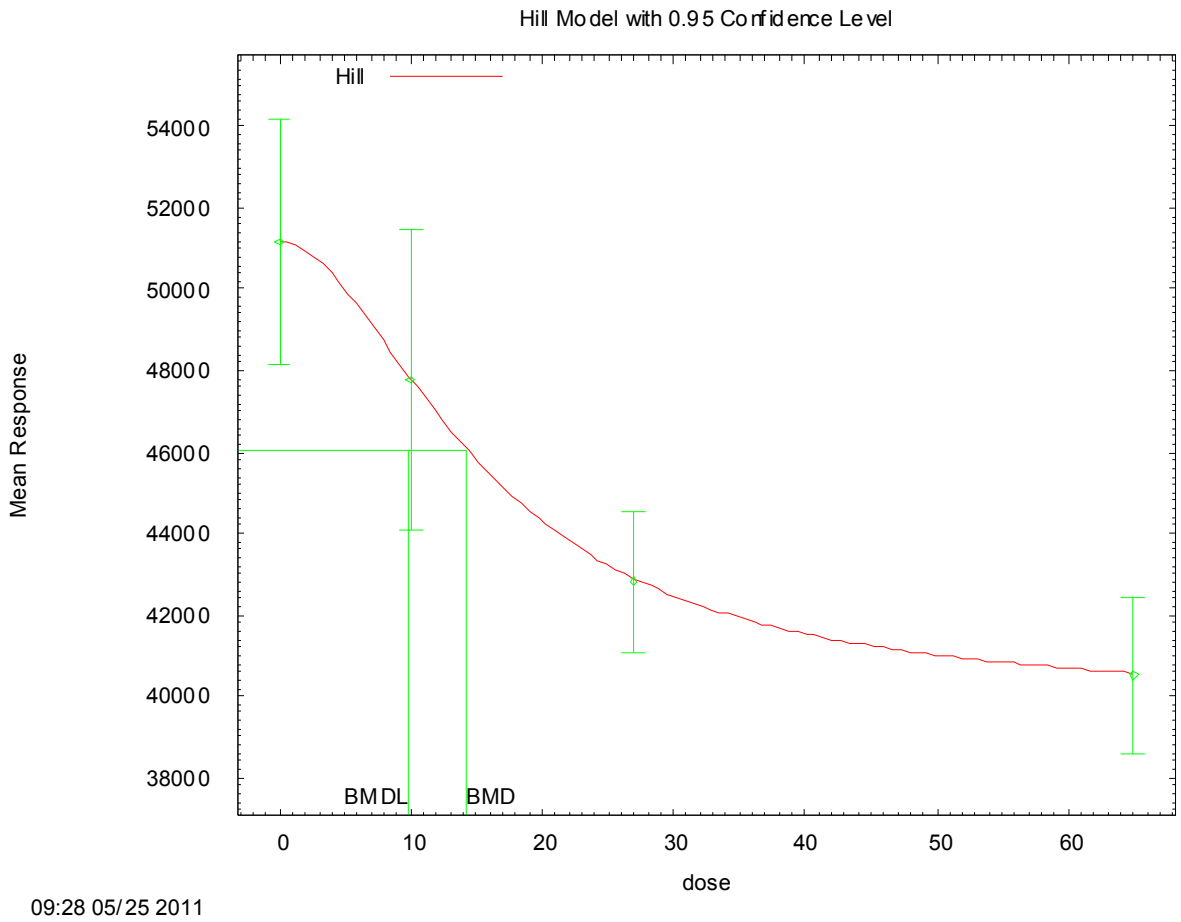



```
Hamada (1993b)
mouse oncogenesis
study with
carbaryl
Hemangiosarcomas- hemangiomas in males
Multistage-Cancer model, top dose deleted
Risk type: "Extra" risk

Multistage Cancer Slope Factor  =   0.00971886

=====================================================================
         Multistage Cancer Model. (Version: 1.10;  Date: 02/28/2013)
         Input Data File:
C:/Usepa/BMDS240/Data/sandbox/msc_hemangio_rubin_bmr05_Opt.(d)
         Gnuplot Plotting File:
C:/Usepa/BMDS240/Data/sandbox/msc_hemangio_rubin_bmr05_Opt.plt
                                                  Wed Aug 07 10:02:06 2013
 ====================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
```

```
 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 = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0548548
                        Beta(1) =   0.00461166
                        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

                                                              95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0516854            *                *                  *
        Beta(1)       0.00498617            *                *                  *
        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         -58.402         2       1.56173      1          0.2114
  Reduced model        -60.6016         1       5.96091      2         0.05077

           AIC:         120.804


                                 Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0517         3.411     2.000          66       -0.785
    2.2500     0.0623         4.109     6.000          66        0.963
   22.3400     0.1516        10.464    10.000          69       -0.156

 Chi^2 = 1.57      d.f. = 1        P-value = 0.2106
```

```
   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        10.2871

            BMDL =        5.14463

            BMDU =        51.2467

Taken together, (5.14463, 51.2467) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00971886
```

Appendix V. 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[dose] = intercept + v*dose^n/(k^n + 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

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     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: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
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
```

Appendix VI. DPR staff "Toxicity Summary" for carbaryl

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
CARBARYL

Chemical Code # 000105, DPN # 00169
SB 950 # 142

September 14, 1987
Revised 10/25/88, 3/05/90, 7/31/90, 1/24/92, 9/7/93, 12/20/93, 1/28/98, 3/6/98, 6/28/99, 10/22/99, 9/20/00, 1/7/02, 4/12/02, 7/27/04, 5/25/05 and 3/29/2006

I. DATA GAP STATUS

Chronic rat:	No data gap, possible adverse effects
Chronic dog:	No data gap, no adverse effect
Oncogenicity rat:	No data gap, possible adverse effects
Oncogenicity mouse:	No data gap, possible adverse effects
Reproduction rat:	No data gap, no adverse effect
Teratology rat:	No data gap, no adverse effect
Teratology rabbit[c, d]:	No data gap, no adverse effect
Gene mutation[a]:	No data gap, no adverse effect
Chromosome mutation[a]:	No data gap, possible adverse effects
DNA damage[a]:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time[b]

Note, Toxicology one-liners are attached
** indicates acceptable study.
Bold face indicates possible adverse effect.
File name: T060329
Original Toxicology Summary prepared by F. Martz, 9/14/87; revised by J. Gee, 10/88, 3/90, 7/31/90 and 1/24/92. Updated by Kellner, 9/7/93 and 12/20/93. Revised by Gee, 1/28/98, 3/6/98, 6/28/99, 10/22/99, 9/20/00, 1/7/02, 4/12/02, 7/27/04, 5/25/05 and 3/29/06.

[a] The data base for genotoxicity on file at DPR may not coincide with that of EPA. Collectively, EPA judged the studies to demonstrate a weakly positive response.
[b] There is an acceptable developmental neurotoxicity study on file with no adverse developmental effects reported. There are an acute and a subchronic neurotoxicity study in the rat on file.

[c] Data gap previously filled with multiple studies. See T990628.
[d] There are two studies with dogs on file that show possible adverse effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

RAT CHRONIC

169 - 099 000719 "Chronic Oral Feeding of SEVIN to Rats." (No author, Mellon Institute of Industrial Research, University of Pittsburgh, Report # 21-88, 10/6/58) Sevin, purity not provided, was fed in the diet for 24 months at 0 (ground Purina Laboratory Chow Meal), 0.005 (50), 0.01 (100), 0.02 (200), and 0.04 % (400 ppm) with 20 CFN rats per sex per group. Interim sacrifices were performed at 6 months (four per sex per group at 0, 0.02 (200), and 0.04 % (400 ppm), 9 months (four per sex per group at 0, 0.02 (200), and 0.04 %), and 12 months (six or 8 per sex per group). At the high dose, decreased body weight gain in males, "cloudy swelling" of kidney tubules at 1 year sacrifice, and "cloudy swelling" of central hepatic cords at two years were noted. The changes are equivocal. Chronic NOEL = 200 ppm, NOAEL = $\geq$ 400 ppm. Uncorrectable deficiencies. UNACCEPTABLE and not upgradeable (insufficient numbers at termination, adequacy of high dose not demonstrated, incomplete necropsy and histopathology). (J. Schreider, 5/9/85 and F. Martz, 5/5/87). Re-examined by Green and Gee, 1/24/92.
EPA One-liner: Systemic NOEL = 200 ppm, LEL = 400 ppm (HDT; decreased weight gain in males, kidneys - cloudy swelling of convoluted and loop tubules, liver - cloudy swelling of hepatic cords about central vein). Core grade: minimum for chronic study, supplementary for oncogenicity study.

154, Tab C, Section II, pp 1-3: Rebuttal to #000719 above. Study cannot be upgraded because of uncorrectable deficiencies, i.e., insufficient group size, no feed analysis, no ophthalmoscopic examinations, no evidence of MTD. Based on these considerations, it would be useless to address the rebuttal point by point. No status change. F. Martz, 8/7/87.

157 & 158, 050433 & -34: Supplemental information to #000719 above consisting of copies of laboratory notebooks and pathology records.

Note: The final report from a recently completed combined chronic/oncogenicity study in rats (HWA Study #656-139) has been submitted by Hazleton and has been found to be acceptable by DPR (see -271:126241 under combined rat), thus filling the chronic rodent data gap. A package from the interim sacrifice of this study (-246:112037) and unaudited histopathological findings from the terminal sacrifice (-261:119579) have also been submitted. Kellner, 12/20/93.

DOG CHRONIC

** 169 056429 "One-year Oral Toxicity Study in Beagle Dogs with Carbaryl Technical." (Hazleton (VA), 3/18/87) Technical carbaryl, 99% pure, was fed at 1250, 400, 125, or 0 ppm in the diet (about 34, 11, or 3.6 mg/kg/day) to 6/sex/dose for 1 year. Cholinesterase inhibition >25% at 1250 and 400 ppm, ChE NOEL = 125 ppm; neutrophilia, slightly increased inorganic phosphorus, and decreased serum albumin at 1250 ppm, not regarded to be adverse effects by reviewer, NOEL = 400 ppm; no clinical signs or organ toxicity, no adverse effects, toxicologic NOAEL = 1250 ppm (HDT). COMPLETE and ACCEPTABLE. F. Martz, 5/19/87.
EPA One-liner: No one-liner on file.

169-099 000718 "Chronic Toxicity of SEVIN for Dogs." (Mellon Institute, report #21-89, 10/1/58) Technical carbaryl was given by oral capsule at 7.2, 1.8, 0.45, or 0 mg/kg/day (approximately equivalent to 400, 100, 25, or 0 ppm in the feed), 5 days/week for 1 year to 3-4 Cocker or Basenji hybrids/level. "… Cloudy swelling of the convoluted and loop [kidney] tubules; sudanophilic dust in the glomeruli..." at 7.2 mg/kg, not regarded by lab to be degenerative change, significance questionable. No other effects, major deficiencies, insufficient information. UNACCEPTABLE and not upgradeable. J. Schreider, 5/10/85.
EPA One-liner: Systemic NOEL = 1.8 mg/kg, LEL = 7.2 mg/kg (diffuse cloudy swelling of proximal convoluted tubule). Core grade: supplementary.

154, Tab C, Section III, pp 3-4; Rebuttal to #000718 above, has no useful information on which to upgrade Mellon Institute study #21-89. Moot because repeat study (#056429 above) was accepted. F. Martz, 5/19/87 (no worksheet).

SUBCHRONIC, DOG

169-239 98146 “Subchronic Toxicity Study in Dogs with Carbaryl Technical.” (N. N. Hamada, Hazleton Laboratories America, Inc., Vienna, Virginia; Report # HLA 656-152; 8/5/91 [completed 3/28/91]). Carbaryl Technical (99.3%, Lot # 87191); 6 dogs/sex/dose; 0, 20, 45, 125 ppm in the diet. Observations: No mortalities due to test article were observed. No significant changes in bodyweight gain, total food consumption, food utilization, clinical observations, ophthalmic changes, or gross pathological changes considered treatment related were observed at any treatment level. Statistically significant decreases in plasma cholinesterase were seen in the 20, and 125 ppm males during Week 2 but were considered incidental. No significant inhibition of erythrocyte or brain cholinesterase levels were seen. NOEL (M/F) >125 ppm (M: 3.83 mg/kg/day, F: 4.11 mg/kg/day; based on no treatment related effects at high dose treatment); Supplemental (length of treatment, limited parameters measured, no histopathology, dose selection not justified: too low) (Miller, 1/21/98)

COMBINED RAT

** 169 - 271 126241 Hamada, N. "Combined Chronic Toxicity and Oncogenicity Study with Carbaryl Technical in Sprague-Dawley Rats" (Hazleton Washington, Inc. (HWA), HWA Study No. 656-139, 9/7/93). Carbaryl technical (lot #12-CNG-32, purity 99%) was administered in the feed at concentrations of 0, 250, 1500, and 7500 ppm to 90 Sprague-Dawley rats/sex/group (control and high-dose) and 80 rats/sex/group (low- and mid-dose) for 104 weeks. Ten

animals/sex/group were sacrificed for clinical pathology evaluation after 26, 52, 78 and 104 weeks; an additional 10/sex (control and 7500 ppm) were sacrificed at week 57 after receiving basal diet from week 53-57 (recovery groups). Body weights and food consumption were significantly lower in high-dose rats during most of the study and in 1500 ppm females at weeks 53 and 105. Cholinesterase (ChE) NOEL = 250 ppm (significant ChE inhibition at the mid- and high dose for erythrocyte and brain ChE and at the high dose for plasma ChE). Nonneoplastic findings: pigment, hyperplasia and eosinophilic foci (liver), foamy macrophages and pneumonitis (lung), vacuolization (pancreas), transitional cell hyperplasia (kidney and urinary bladder), follicular cell hypertrophy (thyroid), nerve degeneration (sciatic nerve/skeletal muscle), decreased leukocytes, unilateral and bilateral cataracts. Systemic (female) NOEL = 250 ppm (male weight effects on occasion at all dose levels). **Possible Adverse Effects**: neoplastic lesions at the high dose level in the urinary bladder (papilloma and carcinoma), kidney (single transitional cell carcinoma in males), liver (adenoma and foci) and thyroid (adenoma and a single carcinoma). ACCEPTABLE. Kellner and Gee, 12/20/93. In the process of developing a risk characterization document, the effect of carbaryl on male body weight was reexamined. There were 3 occasions when the total body weight of males at 250 ppm was statistically significantly lower than controls, (3%), this is of doubtful toxicological significance. Therefore, the NOEL for this study is considered to be 250 ppm as for females in the initial review. (Gee, 5/25/05)

169-246 112037, "Combined Chronic Toxicity and Oncogenicity Study with Carbaryl Technical in Sprague-Dawley Rats." (52-Week Interim Report with 4-Week Recovery) (N. Nicki Hamada, Hazleton Washington, Inc., Vienna, VA., Report #656-139, 12 December 1991). Sevin Technical (Carbaryl), 99.6% purity was used. This is a 52-week interim report (including a 4-week recovery period) for a 104 week study. The test compound was administered in the diet for 52 weeks at 0 (Purina® Certified Rodent Chow® # 5002), 250, 1500 or 7500 ppm with 80 (low and mid-dose) or 90 (control and high dose) Crl:CD BR rats per sex per group. Ten (10) per sex per group were necropsied at week 53. Additionally, following 52 weeks of treatment, 10 per sex each from the control and high dose groups were designated recovery animals and were placed on the basal diet for 4 weeks. These rats were sacrificed and necropsied at week 57. Reduced body weights were noted throughout the study at 1500 (females, 3% to 7% reduction) and 7500 (both sexes, 15% to 38% reduction) ppm. Increased relative (to terminal body weight) liver and kidney weight ratios were indicated for both sexes at 1500 and 7500 ppm. Hepatocellular intracytoplasmic hyaline inclusions were noted in 1 and 4 high dose males respectively at the unscheduled and interim (week 53) sacrifices. Histopathology of recovery animals (week 57 sacrifice) showed the absence of this finding, suggesting reversibility. **Adverse effects are not indicated**. Possible Chronic NOEL = 250 ppm (bodyweight reduction). ChE NOEL = 250 ppm (based on plasma, RBC and brain ChE inhibition at 1500 and 7500 ppm). **Unacceptable**, this 52-week interim report does not satisfy chronic data requirements in the rat for a food-use active ingredient. It is considered supplemental information, pending receipt of the final report. (H. Green, 1/15/92, and Gee, 1/23/92)
Note: Final report has been submitted and is acceptable; see 169 -271:126241.

169-261 119579 [Addendum to -246:112037] Hamada, N. "Chronic Toxicity/ Oncogenicity Study with Carbaryl Technical in Rats Preliminary Data" (HWA Study No. 656-139, 11/3/92). Carbaryl technical was administered in the feed to Sprague-Dawley Rats at 0, 250, 1500 or 7500 ppm; this submission concerns preliminary unaudited neoplastic findings from terminally sacrificed rats and intercurrent deaths from Hazleton study #656-139. **Possible Adverse Effects:** Increased incidence of bladder (males and females), thyroid (males) and hepatocellular (females) neoplasia. Increased sciatic nerve and skeletal muscle degeneration in the high-dose group. Kellner and Gee, 8/31/93.

RAT ONCOGENICITY

Also See Combined Rat.

EPA One-liner: Rat Oncogenicity study on file (Carpenter et al., 1961, J. Agriculture and Food Chemistry, 9:30-39.); Core Supplementary.

MOUSE ONCOGENICITY

**** 267 123769** Hamada, N. "Oncogenicity Study with Carbaryl Technical in CD-1® Mice." (Hazleton Washington, Inc. (HWA), HWA Study No. 656-138, 5/20/93). Carbaryl technical (lot #87191, purity 99.3%) was administered in the feed at concentrations of 0, 100, 1000 and 8000 ppm to 80 CD-1® mice/sex/group for 104 weeks. Ten animals/sex/group were sacrificed at interim (week 52). Non-neoplastic findings included increased incidence of intracytoplasmic (protein-like) droplets in the superficial transitional epithelium of the urinary bladder and increased hematopoiesis and pigment in the spleen (high-dose). High-dose mice appeared unthrifty (hunched, languid, thin, urine stains, rough coat and opaque eyes) and showed reduced body weight (18%) and food consumption (22%). Lung and ovary weight were reduced and liver weight was elevated for the high dose groups. **Systemic NOEL = 100 ppm** (from effects seen in the urinary bladder). Cholinesterase activity (RBC-CHE and BR-CHE) showed significant decreases in the mid- and high-dose groups; **ChE NOEL = 100 ppm. Possible adverse effects:** Increased hemangioma/hemangiosarcoma in all male dose groups and high-dose females; increased renal tubular cell adenoma and carcinoma in high-dose males and hepatocellular adenoma and carcinoma in high-dose females. Unilateral or bilateral posterior lens cataracts at high-dose. ACCEPTABLE. (Kishiyama, Kellner and Gee, 8/23/93).

254 116336 [Addendum to -267:123769] Hamada, N. "Oncogenicity Study with Carbaryl Technical in Mice Preliminary Data." (Hazleton Washington, Inc., HWA Study No. 656-138, 7/21/92). Carbaryl technical, purity 99.3%, was administered in the feed at levels of 100, 1000 and 8000 ppm to 80 CD-1® mice/sex/group for 104 weeks; this submission reports preliminary neoplastic findings from terminally sacrificed mice and intercurrent deaths. **Possible Adverse Effects:** There were increased incidences of renal (high-dose males), hepatocellular (high-dose females), and vascular (males and females) neoplasia. Also reported was increased incidence of unilateral and bilateral cataracts in high-dose male and female mice. Supplemental Data. (Kellner and Gee, 8/6/93.)

169-247 112020 Partial duplicate of -267:123769. Contains data and analysis up to and including the 52-Week interim sacrifice. No worksheet. (Kellner, 9/7/93.)

169 – 400 177757 E. Debruyne "Carbaryl: 52-Week Toxicity Study in the CD1 Mouse Target Organs Cell Cycling Assessment." (Rhone-Poulenc Agro, Study SA 97529, 12/02/98) Paraffiin blocks containing tissues were obtained from study HWA 656-138 [Record 123769, Hazleton Labs, 1993]. Female livers and male kidneys of mice from the 8000 ppm treatment group, 10/group, sacrificed after 52 weeks of exposure, were compared with untreated control mice. A section of rat duodenum was used as the positive control for immunohistochemical staining for proliferating cell nuclear antigen (PCNA) to assess cell cycling. Deparaffinized tissue sections were reacted with PCNA, amplified with a secondary antibody, submitted to a complex of streptavidin-peroxydase and reacted with the chromogen aminoethylcarbazol.

PCNA-positive cells had red-stained nuclei and non-proliferating nuclei were blue. 1000 cells were evaluated per section of liver and kidney. For male kidneys, PCNA-positive renal cortical tubular cells had a mean of 1.20 ± 1.75 per 1000 cells (range of 0 to 4) while treated tissue had 3.90 ± 2.18 (range of 1 to 7). For female hepatocytes, control mean was 4.60 ± 7.68 (range of 0 to 23) and treated, 8.33 ± 3.84 (range of 2 to 13). The results were interpreted as of uncertain toxicological significance for male kidneys and not significant in female livers, based on the range of variability and the small difference in males and that all treated female values were within control range. Therefore, overall, increased cell cycling of apparent target tissues was not clearly demonstrated by this approach. Positive control data from the rat were not, however, included in the report. Supplemental study with no worksheet. (Gee, 3/28/06)

169 - 099 000717 (with rebuttal and supplemental information in -154 and -161, 050437) "Results of Eighty Weeks of Inclusion of SEVIN in the Diet of Mice." (Mellon Institute, report #26-53, 6/11/63) Technical carbaryl, 99.8% pure, was fed in diet at 400, 100, or 0 ppm to CD-1 mice, 48/sex/level for 80 weeks. There was approx. 50% mortality at 80 weeks, in all groups, ½ of these autolyzed or cannibalized; 12/sex/level sacrificed at 80 weeks with no explanation of the survivors' fates; latter found only in supplemental information in #050437; no onco effects, but

study generated little useful information. UNACCEPTABLE and not upgradeable J. Schreider, 5/10/85 and F. Martz, 8/7/87)

EPA One-liner: Negative - dietary at 400 ppm (HDT)/day/2 yr. Core grade: supplementary.

169-154, Tab C, Section V, pg. 5: Rebuttal of #000717 above. Study cannot be upgraded. Twelve/sex/level were interim sacrificed at 80 weeks and the study terminated at 2 years. Results of the terminal sacrifice are not given in the report. Approximately 50% of the mice died by 80 weeks, and one-half of these lost to autolysis and/or cannibalism; only 2-10 mice/level examined from interim sacrifice through termination; the tissue inventory is incomplete. Based on these considerations, the study is not upgradeable and the rebuttal will not be discussed further. F. Martz, 8/7/87.

169-161, 050437: Supplemental information to #000717 above, having the following:

Tab A: Copy of laboratory notebook 806. Individually lists by animal number, the calendar date and fate, age at fate, clinical observations, and gross necropsy observations. Note that this provides the first indication that the study actually went beyond 80 weeks. The report stated that 12/sex/level were sacrificed at 80 weeks with no explanation of the survivors' disposition.

Tab B: Copy of laboratory notebook 807. Contains diet room records showing amounts of carbaryl and feed used for each batch of feed mix.

Tab C: Consultant pathologist's second opinion of liver findings. No new effects noted. The slight increase in hepatocellular nuclear polyploidy noted originally at 80 weeks was re-diagnosed as being "...present in the livers of all mice examined…." There was no change in the hepatocellular tumor incidence.

Tab D: Pathology report from 80 week sacrifice through termination.

Tab E: Pathology report from first death through 80 week sacrifice.

F. Martz, 8/7/87.

MOUSE SUBCUTANEOUS ONCOGENICITY

169-023, 038178 (with rebuttal and additional information in -154 and -160, 50436) "Mammalian Toxicity of 1-Naphthyl-N-methylcarbamate (SEVIN Insecticide)." Mellon Institute, in J. Agr. Food Chem., 9:30-39 (1961); technical carbaryl in 0.25% agar by subcutaneous injection once weekly to 3 month old A/J or C3H males, 10 or 0 mg/mouse, 30/level, and 30 untreated controls, for 5 months with gross examination for lung masses at 8 months of age. UNACCEPTABLE, contains no useful information. (J. Schreider, 5/6/85 and F. Martz, 5/5/87)
EPA One-liner: Negative - subcutaneous injection of 5% (10 mg) agar dilution (HDT) once/wk/20 wk. Core grade: supplementary.
169-154, Tab C, Section IV, pg. 5; Rebuttal of #038178 above. Study cannot be upgraded and rebuttal will not be further addressed. No status change. F. Martz, 5/5/87.
169-160, 050436; Copies of 4 laboratory notebook pages for #038178 above.

RAT REPRODUCTION

** 169 - 410 182115 Tyl, R. W., C. B. Myers and M. C. Marr " Two-generation reproductive toxicity evaluation of Carbaryl (RPA007744) administered in the feed to CDOL 226 \f "Symbol" \s 11 (Sprague-Dawley) rats." (Research Triangle Institute, RTI 65C-07407-400, 5/24/2001) Technical grade carbaryl, 99.1%, was fed in the diet at 0, 75, 300 or 1500 ppm to 30/sex/group CD® Sprague Dawley rats for 1 litter per generation, two generations. At 1500 ppm, there were decreased body weight and food consumption in F0 and F1 parental animals with smaller effects at 300 ppm. In offspring, there were lower body weights, delay in vaginal opening and preputial separation (measured in F1 pups only), increased mortality in F1 and F2 pups at 1500 ppm with an increase in mortality in F2 pups at 300 ppm during lactation, especially pnd 0 - 4 (survival index of 98.3% for controls, 92.0% at 300 ppm - not statistically significant, and 88.9% at 1500 ppm - also not significant). Parental systemic NOEL = 75 ppm; reproductive NOEL = 1500 ppm (no effects); pup NOEL = 75 ppm. No specific adverse reproductive effects. ACCEPTABLE. (Gee, 1/7/02).

169 - 388 170645 “Carbaryl reproductive toxicity: Assessment of data adequacy for hazard assessment and evaluation of potential for increased susceptibility to the young.” (J. P. Rieth, Rhone-Poulenc Ag Company, report no JPR0199, May 20, 1999). The document is an assessment of the results of reproductive and developmental studies in view of FQPA and the need for an additional 10X safety factor and addressed to US EPA. The reproduction studies completed in 1966 and 1972 were discussed and a statement was made that a new reproduction study is in progress and due in December, 2000. SUPPLEMENTAL. (Gee, 9/10/99)

169 - 099 000716 "Results of a Three Generation Reproduction Study on Rats Fed SEVIN in Their Diets." (Mellon Institute report #28-53, 4/20/65) Technical grade carbaryl, 99.8% pure, in the feed at 10, 2.5, or 0 mg/kg/day to 12-20 females/level, males unspecified, (F_{2a}-->F_{3b} for teratology portion); no reproductive effects, but no MTD; UNACCEPTABLE because of numerous deficiencies. J. Schreider, 5/10/85 and F. Martz, 8/6/87.
EPA One-liner: Reproductive, fetotoxic, and maternal NOEL=10 mg/kg (HDT). Core grade: minimum.

169 - 130 037909; Exact duplicate of #000716 above. F. Martz, 8/7/87.

154, Tab C, Section VI, pages. 6-7, Rebuttal of #000716 above. Basically, an explanation of the study design in comparison to a FIFRA guideline study. My response is to quote the registrant's own rebuttal of the teratology portion of this study given in Section VII: "The value of

this study will not be discussed in detail as the dosage levels used...were so much lower than those used in a subsequent study." F. Martz, 8/6/87 (no worksheet).

162, 050438; Additional data included with rebuttal for #000716 above, consisting of photocopies of laboratory notebooks 911, 914, 945, and 951 as well as typed text and tables:
Tab A: Contains the "Diet Room Record" documenting the amounts of carbaryl and feed used for diet preparation;
Tab B: Individual body weights of F_0, F_{1a}, and F_{1b}, and litter records for the F_0 and F_{1a} rats;
Tab C: Body weights and mating records of the F_2 generation;
Tab D: Litter records for pups of the F_2 females;
Tabs E, F, and G: Pathology summaries and raw data for fetuses, weanlings, and 90 day old individuals from the F_3 offspring.
Additional data can not upgrade study. F. Martz, 5/19/87.

169-099 000712 (with rebuttal and additional data in -154, -165, 050441, and -166, 050442, 050955 & 56). "Comparative Study of Dietary Inclusion versus Stomach Intubation on Three-Generations of Reproduction, on Teratology and on Mutagenesis." (Mellon Institute, report #35-65, 8/31/72) Technical carbaryl, 99.6% pure, in feed at 200, 100, 25, 7, or 0 mg/kg/day; by gavage in corn oil at 100, 25, 7, 3, or 0 mg/kg/day; in feed containing corn oil at 100 or 0 mg/kg/day; 5 days/week (m->f); limited->complete histopathology on P_0 and F_{3a} at 21 and 90 days old;
FEEDING RESULTS: MATERNAL - reduced weight gain at 200 mg/kg; NOEL = 100 mg/kg maternal, 200 mg/kg repro;
FEEDING/CORN OIL RESULTS: no effects at 100 mg/kg;
GAVAGE RESULTS: MATERNAL - tremors, mortality, and reduced weight gain at 100 mg/kg; REPRO - decreased litter size at 100 mg/kg; NOEL = 25 mg/kg maternal and repro.
PROBLEMS: excessive postnatal mortality days 4-21 potentially hiding subtle effects, makes otherwise upgradeable study UNACCEPTABLE. J. Schreider, 5/13/85 and F. Martz, 8/7/87.
EPA One-liner for gavage: Reproductive NOEL = 25 mg/kg, fetotoxic LEL = 100 mg/kg (decreased "viable" fetuses), maternal LEL = 100 mg/kg (HDT; decreased weight gain, cholinergic signs, mortality). Core grade: Minimum.
EPA One-liner for feeding: Reproductive and fetotoxic NOEL = 200 mg/kg (HDT), maternal LEL = 200 mg/kg (decreased weight gain). Core grade: minimum.

169 - 130 037913: Exact duplicate of #000712 above.

169 - 154, Tab C, Section X, pages 10-11, Rebuttal of #000712 above. Specific comments are as follows:
A. Rebuttal: "A single daily dose intubation procedure is not considered by the authors to be a proper model for human exposure to pesticide residues in the diet." This concerns our criticism that carbaryl was administered by feed rather than gavage.
Response: We disagree. Humans may consume a carbaryl-laden meal in 10 minutes, whereas rats nibble their feed continuously albeit mostly with the first several hours of darkness.
B. Rebuttal: "Furthermore, the previous...studies using dietary inclusion ...demonstrated no reproductive or teratogenic effects."
Response: Dose levels (25 mg/kg HDT) were insufficient.
C. Rebuttal: "...the purpose of this study was [to compare]...the potential effect on reproduction in rats following daily dietary inclusion or gastric intubation. To attempt to compare the procedure used to the current FIFRA guidelines is inappropriate."
Response: We agree. Were it not for excessive postnatal mortality in particular, the study could be acceptable to fill the data gap.

D. Rebuttal: "The results of this study were, and are, very significant. Daily gastric intubation of carbaryl resulted in maternal cholinesterase inhibition and mortality, as well as some effect on reproduction but the appropriate potential hazard route of administration, dietary inclusion, was without significant deleterious effect."

Response: We disagree that dietary inclusion is the most appropriate route of administration for reasons stated above.

E. Comment: The respondent seems to have missed the overwhelming significance of this study: the complete absence of teratogenicity with only minimal fetotoxicity or reproductive toxicity within the parameters covered at a gavage-administered dose level clearly producing significant maternal toxicity. That alone supports the relative "safety" of carbaryl to the developing organism. F. Martz, 8/6/87.

169 - 165, 050441 Additional data submitted with rebuttal for rat reproduction study with teratology and dominant lethal components (Mellon report #35-65, CDFA #169-099, #000712 and 027204), consisting of photocopies of laboratory notebooks;

Tab A: Individual body weights of F_0 rats and identification of male-female mating pairs for F_{1a}, F_{1b}, and F_{1c} offspring;

Tab B: "Diet Room Record" documenting usage of carbaryl and feed for diet preparation from start to 8/9/71, continued in Tab J;

Tab C: Individual body weights of oral gavage F_0 parents and F_{1a} offspring from weaning through gestation of F_{2a}, and clinical observations.

Tab D: Contains body weight data for what appears to be a 4 week pilot study otherwise unidentified;

Tab E: Litter records for F_0 matings;

Tab F: Individual body weights and mating-pair records for feed-treated F_{1a};

Tab G: Contains F_{1a} body weight information continued from Tab C, and F_{2a} body weights;

Tab H: Litter records for F_{1a} matings;

Tab I: Body weight records for F_{2a} rats from weaning through gestation of F_{3a} offspring;

Tab J: Contains diet room records continued from notebook in Tab B. F. Martz, 5/11/87.

169 - 166 Additional data for rat reproduction study with teratology and dominant lethal portions (Mellon report #35-65, CDFA #169-099, 000712 and 027204), consisting of photocopies of laboratory notebooks or typed reports;

Record # 050955:

Tab A contains litter records for F_{2a} dams which delivered, consisting of birth dates, individual pup weights and the number of live or dead pups in the F_{3a} generation;

Tab B contains similar litter records for F_1 dams which delivered; Tab C contains individual body weight records for the gavage-treated F_{3a} groups;

Tab D contains similar information for feed-treated F_{3a} groups;

Record # 050442:

Tab E contains mating records for F_{2a} parents to produce the F_{3b} for the teratology portion of study;

Tab F contains individual F_{2a} dam sacrifice records for teratology portion of study, consisting of sacrifice calendar dates, the number of live or dead pups or resorption sites, the number of pups with gross anomalies and descriptions of observed anomalies, and litter weights;

Tab G contains a pathology report for an unidentified 1 month rat study at 150, 75, or 0 mg/kg/day via the feed;

Tab H contains pathology report for the sacrificed F_{3a} rats from the feed-treated groups, continued in Tab M for gavage-treated groups;

Tab I contains pathology report dated 3/21/72 for unidentified rat study using dietary dose levels of 200 or 0 mg/kg/day;
Tab J contains pathology report also dated 3/21/72 for unidentified rat study using oral gavage dose levels of 100 or 0 mg/kg;
Tab K contains pathology report dated 2/22/72 for teratology portion of reproduction study, with tabulation of skeletal and visceral observations;

Record # 050956:
Tab L contains report of uterine observations from dominant-lethal portion of reproduction study;
Tab M is a continuation of material in Tab H, and contains a pathology report dated 6/23/72 for the sacrificed F_{3a} pups from the gavage-treated groups;
Tab N contains duplicate pathology records for F_{3a} pups sacrificed on day 21 as well as records for F_{3a} rats sacrificed at 90 days of age;
Tab O contains pathology report dated 8/26/72 for F_{3a} rats sacrificed at 90 days of age, appears to be duplicate of some material in Tab N.
F. Martz, 5/19/87.

179 059543 "The Effect of Carbaryl (Sevin) on Reproduction of the Rat and the Gerbil." (Food and Drug Administration, Division of Pesticide Chemistry and Toxicology, DC, publication in Toxicol. Appl. Pharmacol. 19: 202-216 (1971), accepted 9/4/70, T. Collins et al.) Technical carbaryl, 99%, was fed in the diet to Osborne-Mendel rats at 0, 2000, 5000 or 10,000 ppm, 20/sex/group, three generations, two litters per generation or to Mongolian gerbils at 0, 2000, 4000, 6000 or 10,000 ppm, three generations, two litters. NOEL for reproductive effects < 2000 ppm (LDT) in both species with reduced weaning weights at all doses, decreased viability, survival, weaning indices at 5000 and 10,000 ppm in the rat with no animals at 10,000 for the second F1 mating or the F2 matings. No abnormalities reported; parental NOEL in the rat appeared to be 5000 ppm from the text; effects similar in the gerbil; no microscopic pathology included in the report; insufficient information to evaluate parental effects. Unacceptable, not upgradeable (no reproductive NOEL - all doses too high. (Gee, 9/30/88)

Previous summary: Although no study alone is adequate, the collective data provide sufficient information. The 1971 study indicated a possible adverse effect on reproduction at a dose not obviously toxic to the parental animals from the report. The publication by Collins et al., however, contains insufficient information for independent assessment of the parental toxicity. The later (1972) study, Record # 000712 and supplements, demonstrated a reproduction NOEL of 200 mg/kg in the feeding portion and a maternal NOEL of 25 mg/kg with no adverse developmental effect. Excessive postnatal mortality was seen in all groups, including the control so it was not a clear treatment effect. Since this study is much more complete and establishes NOELs, the conclusion is that there is no adverse reproduction effect without parental effects. From the 1972 study, it is likely that cholinesterase was markedly inhibited in the parental animals in the study by Collins et al. The collective data fill the data gap. Gee, 10/25/88.

NOTE: Record No. 170645 in 169-388 contains a statement that a new reproduction study is in progress with a due date of December, 2000. Gee, 10/1/99. This study was submitted in 169 - 410, record no. 182115 - see 1-liner above. Gee, 1/7/02.

RAT TERATOLOGY

** 169-383 166125 " Carbaryl: Developmental toxicology study in the rat by gavage." (M. Repetto-Larsay, Study Director, Rhone-Poulenc Agro, Study SA 98070, October 21, 1998). Pregnant rats (Crl:CD(SD)BR), 25 per group, were given carbaryl (lot 208 115 110, 99%), at doses of 0 (0.5% methylcellulose 400), 1, 4 or 30 mg/kg/day by gavage, days 6 - 20 of gestation. Fetuses were given an external examination and approximately half were examined for visceral changes and half for skeletal effects. At 30 mg/kg/day, 18/25 dams had increased salivation within 20 minutes of dosing, disappearing within 1 hour and observed primarily between days 14 to 20 of gestation. In addition, maternal body weight was reduced statistically significantly at 30 mg/kg and fetal body weight was also reduced at 30 mg/kg. Maternal NOEL = developmental NOEL = 4 mg/kg/day (body weights, clinical signs in dams). ACCEPTABLE. No adverse effect. (Gee, 2/22/99)

169 - 099 000716 (With rebuttal and additional information in -154 and -162, 50438) "Results of a Three Generation Reproduction Study on Rats fed SEVIN in Their Diets," teratology of F_{3b}; Mellon Institute, report #28-53) 4/20/65; technical grade carbaryl, 99.8% pure, in the feed at 10, 2.5, or 0 mg/kg/day to 17-18 pregnant rats/level (F_{2a} of reproduction study) from prior to mating to sacrifice day 18-21; no soft tissue exams; no developmental toxicity, but no maternal MTD. UNACCEPTABLE and not upgradeable. J. Schreider, 5/10/85 and F. Martz, 5/6/87.
EPA One-liner: None in file.

169 - 154, Tab C, Section VII, pg. 8, Rebuttal of #000716 above: To quote: "The value of this study will not be discussed in detail as the dosage levels used...were so much lower than those used in a subsequent study." Agree. F. Martz, 5/6/87.

169 - 162 050438 Additional data included with rebuttal for #000716 above, consisting of photocopies of laboratory notebooks 911, 914, 945, and 951 as well as typed text and tables:
Tab A: Contains the "Diet Room Record" documenting the amounts of carbaryl and feed used for diet preparation.
Tab B: Individual body weights of F_0, F_{1a}, and F_{1b}, and litter records for the F_0 and F_{1a} rats;
Tab C: Body weights and mating records of the F_2 generation ;Tab D: Litter records for pups of the F_2 females;
Tabs E, F, and G: Pathology summaries and raw data for fetuses, weanlings, and 90 day old individuals from the F_3 offspring. Additional data do not upgrade study although they indicate good documentation procedures. F. Martz, 5/19/87.

169 - 099 000715 (With rebuttal and additional information in -154 and -163, 50439) "Evaluation of the Teratogenic Potential of Insecticide SEVIN in Rats." (Mellon Institute, report #29-49, 7/28/66) Technical grade carbaryl, 99.7% pure, in the feed, was adjusted to give 500, 100, 20, or 0 mg/kg/day to 3 groups with 12/level each. The groups were treated: (1) throughout pregnancy or until weaning, (2) gestation days 0-7, or (3) gestation days 7-15 with one-half sacrificed days 19-21, the other half allowed to deliver with termination 21 days postpartum; gross and skeletal exams only.
MATERNAL: dose-related weight gain reduction, severest in group 1 (dosing throughout pregnancy);
DEVELOPMENTAL: none - no fetotoxicity or skeletal malformations; POSTNATAL: reduced live litter size and postnatal survival at 500 mg/kg (not considered a teratogenic effect);
MATERNAL NOEL = 20 mg/kg; FETOTOXIC NOEL = 100 mg/kg (based on reduced liveborn litter size); TERATOGENIC NOEL = 500 mg/kg (soft tissue exclusive).
UNACCEPTABLE and not upgradeable. J. Schreider, 5/10/85 and F. Martz, 8/6/87.
EPA One-liner: Teratogenic NOEL > 500 mg/kg (HDT), maternal LEL = 500 mg/kg (decreased

weight gain), fetotoxic LEL = 500 mg/kg (mortality). Core grade: minimum.

169 - 130, 037910 Exact duplicate of #000715 above.

169 - 154, Tab C, Section VIII, pg. 8, Rebuttal of 29-49 above: Points are addressed as listed:
A. Rebuttal: "This design is more informative that [sic] the cook-book one in the guidelines..."
Response: We agree that the study design would provided more useful information overall than the standard Segment II type of teratology study, if all relevant parameters were covered, which they weren't.
B. Rebuttal: "It is notable that the dosage levels are remarkable and appropriate..."
Response: We agree. That the high dose level was a MTD is documented by the severely reduced body weight gain during gestation in that group.
C. Rebuttal: "The frequency of weighings and food consumption measurements, and these results for individual animals and dosage groups, may be found in the supplied Mellon Institute notebook 984 and 987."
Response: Located in -063, 50439.
D. Rebuttal: "The reviewer remarked: 'Sex and weight of fetuses not recorded.' The results are summarized...in Table 29-88..."
Response: Table gives body weight of pups at weaning only. The fetal weights recorded at hysterectomy are still unaccounted for.
Comment: There is no evidence that soft tissue examinations were done. Therefore, the potential to cause visceral malformations remains unanswered by the data collected in this study.
Conclusion: The study is incomplete, unacceptable, and not upgradeable. F. Martz, 5/6/87.

169 - 163 050439 Additional data for rat teratology study #29-49, (CDFA #169-099, 715), consisting of photocopies of laboratory notebooks 911, 984, and 987:
Tab A contains "Diet Room Records" documenting the amounts of carbaryl and feed used in preparing diet blends.
Tab B contains individual female body weight values.
Tab C contains uterine examination observations of dams killed on day 20, and litter records on dams allowed to deliver and wean their offspring. Records consist of total pups in utero and dead or resorbed pups, anomalous observations, the dates of sacrifice or weaning, the number of pups born, on day 4 and 21, the individual pup weights according to sex on day 21, and any abnormalities.
While this volume contains good documentation of experimental conduct, it does not upgrade the report, mainly due to lack of feed analysis and soft tissue examinations. F. Martz, 5/12/87.

** 169 - 099 027204 (With rebuttal and additional information in -154 and -166, 50442) "Comparative Study of Dietary Inclusion versus Stomach Intubation on Three-Generations of Reproduction, on Teratology and on Mutagenesis. " (Mellon Institute, report #35-65, 8/31/72). Technical carbaryl, 99.6% pure, in feed at 200, 100, 25, 7, or 0 mg/kg/day; by gavage in corn oil at 100, 25, 7, 3, or 0 mg/kg/day; in feed with corn oil equivalent at 100 or 0 mg/kg/day; to 6 month F_{2a} males and females 5 days/week (M->F) before and during mating/gestation; F_{3b} offspring examined;
FEEDING RESULTS: MATERNAL - decreased weight gain at 200 mg/kg, FETAL - incomplete ossification at 200 mg/kg; NOEL = 100 mg/kg maternal and fetal;
FEEDING/CORN OIL RESULTS: no effects at 100 mg/kg;

GAVAGE RESULTS: MATERNAL - cholinergic signs, mortality and reduced weight gain at 100 mg/kg, FETAL - reduced live litter size, more litters with resorptions, and incomplete ossification at 100 mg/kg; NOEL = 25 mg/kg maternal and fetal. Originally unacceptable (J. Schreider, 5/20/85) but upgraded to ACCEPTABLE (with major deviations). F. Martz, 5/87.
EPA One-liner for gavage: Teratogenic NOEL > 100 mg/kg (HDT), maternal LEL = 100 mg/kg (decreased weight gain, cholinergic signs, mortality), fetotoxic NOEL = 100 mg/kg (decreased live fetuses). Core grade: minimum.
EPA One-liner for feeding: Teratogenic NOEL > 200 mg/kg (HDT), maternal LEL = 200 mg/kg (decreased weight gain), fetotoxic NOEL > 200 mg/kg. Core grade: minimum.

154, Tab C, Section XI, pg. 11, Rebuttal of above study. Registrant's rebuttal and CDFA's response is similar to that given in "REPRODUCTION RAT, 35-65" below. We agree with rebuttal. In spite of major deviations from guidelines, the data appear to have been gathered in a manner scientifically valid and with good documentation. Total "weight of evidence" supports the absence of teratogenic potential. In my opinion, no new significant information would be gained from a study conducted according to current guidelines. Therefore, the teratology data gap is filled. F. Martz, 5/6/87.
166, 050422; Additional data for #027204 above; see -166 entry under "REPRODUCTION RAT (Mellon Report #35-65)."

169 - 131 to 133, 037925-27 "Teratology Study - SEVIN, Vitamin A, Aspirin and Malathion." (Litton, 6/23/72.) Technical carbaryl, 99.6% pure, was fed at 7000, 4000 or 0 ppm (approximately 375 or 200 mg/kg) to timed pregnant Sprague-Dawley females (Flow Labs; plug day=0), 20/level, days 6-15 with sacrifice on day 18, 1/3 fetuses visceral, remaining skeletal. No developmental toxicity, maternal weight gain can not be assessed from data presented, but data "...do not appear to show any evidence of maternal toxicity." NOEL>7000 ppm (approximately 375 mg/kg). F. Martz, 12/11/85 and 8/7/87.
EPA One-liner: NOEL = 375 mg/kg (HDT). Core grade: supplementary.

RABBIT TERATOLOGY

** 169-389 170646 “Developmental toxicity evaluation (with cholinesterase assessment) of carbaryl administered by gavage to New Zealand White rabbits.” (R. W. Tyl, M. C. Marr and C. B. Myers, Research Triangle Institute, RTI No. 65C-7297-200/100, 6/3/99.) New Zealand White rabbits, 22/dose group, were given carbaryl (batch 208115110, 99% purity) by gavage at 0 (0.5% aqueous methylcellulose), 5, 50 or 150 mg/kg body weight/day on days 6 through 29 of gestation. Animals were sacrificed on gestation day 30. Dose selection was based on a range-finding study with 100 mg/kg as the high dose. Plasma cholinesterase was inhibited to 41% of control and RBC cholinesterase was 80.1% of control (not statistically significant) at 100 mg/kg. In the definitive study, body weight gain was reduced at 150 mg/kg, being 47% of control. Total body weight, however, was not significantly lower. Plasma cholinesterase was 46% and 32% of control at 50 and 150 mg/kg/day, respectively. Red blood cell cholinesterase was 81% and 73% of control at these doses. These values were statistically significant. At 150 mg/kg, fetal body weight was reduced, being 90% of control. There were no other developmental affects reported as related to treatment by the authors. There were two fetuses in two litters with agenesis of the gall bladder(10%, p = 0.27 by Fisher’s Exact) and 4 fetuses from three additional litters reported as having gall bladders “half normal size.” These incidences were compared with 0/18 for the concurrent controls. The historical control incidence for agenesis of the gall bladder included in the report indicated 1/187 litters (0.53%). Maternal NOEL = 5 mg/kg (cholinesterase inhibition). Developmental NOEL = 50 mg/kg (reduced fetal body weight)

No adverse effects. ACCEPTABLE. (Gee, 9/24/99)

Report not in CDFA file, but study by Robens (FDA), "Teratologic studies of carbaryl, diazinon, norea, disulfiram, and thiram in small laboratory animals," was published in Toxicol. Appl. Pharmacol. 15: 152-163 (1969).
EPA One-liner: Teratogenic, fetotoxic, and maternal NOEL all > 200 mg/kg by oral gavage (HDT). Core grade: minimum.

Report not in CDFA file, but study by Murray et al, "Teratogenic potential of carbaryl given rabbits and mice by gavage or by dietary inclusion," was published in Toxicol. Appl. Pharmacol. 51: 81-89 (1979).
EPA One-liner: Teratogenic, fetotoxic, and maternal NOEL al l > 200 mg/kg by oral gavage (HDT). Core grade: minimum.

MOUSE TERATOLOGY

Report not in CDFA file, but study by Murray et al, "Teratogenic potential of carbaryl given rabbits and mice by gavage or by dietary inclusion," was published in Toxicol. Appl. Pharmacol. 51: 81-89 (1979).
EPA One-liner for gavage: Teratogenic and fetotoxic NOEL>150 mg/kg (HDT), maternal LEL = 150 mg/kg (decreased weight gain, cholinergic signs). Core grade: minimum.
EPA One-liner for feeding: Teratogenic NOEL > 1166 mg/kg (HDT), fetotoxic LEL = 1166 mg/kg (decreased weight), maternal LEL = 1166 mg/kg (decreased weight gain). Core grade: minimum.

TERATOLOGY DOG

169 - 099 000714 "Sevin - Safety Evaluation by Feeding to Female Beagles From Day One of Gestation Through Weaning of the Offspring." (Woodard Res. Corp., 1/22/69) "Sevin technical grade," 99.8% pure, in the feed at 12.5, 5.0, 2.0, or 0 mg/kg/day, gestation day 1 through weaning at 6 weeks of age; increased stillbirths at 12.5 and 5 mg/kg; decreased birth weights and reduced survival to weaning at 12.5 mg/kg; inconclusive treatment-related malformations. UNACCEPTABLE and not upgradeable because of numerous deficiencies. J. Schreider, 5/10/85 and F. Martz, 8/4/87
EPA One-liner: Teratogenic NOEL = 2 mg/kg (LDT), teratogenic LEL = 5 mg/kg - (umbilical hernia, cleft palate, gastrointestinal abnormalities), Maternal NOEL < 2 mg/kg - (dystocia). Core grade: supplementary.

130, 037911: Exact duplicate of #000714 above.

No Record Number. Smalley, H. E., J. M. Curtis and F. L. Earl. “Teratogenic action of carbaryl in beagle dogs.” Published in Toxicology and Applied Pharmacology 13: 392 - 403 (1968) (Division of Pharmacology and Toxicology, Food and Drug Administration, U. S. Department of Health, Education and Welfare) Technical grade carbaryl (lot 5072, 99.9%) was fed in the diet to beagle dogs at 0, 3.125, 6.25, 12.5, 25 or 50 mg/kg/day. Females were mated when in estrus with one male on day 1 and a second male on day 3. Dosing began on a Wednesday between day 3 and day 6 after mating. With the exception of 6 dogs, all were allowed to give birth and the pups weaned at 8 weeks. Following weaning, they were sacrificed and autopsied. The number of females per dose group varied between 16 for concurrent

controls and 8 at the highest dose. There were no clinical signs or differences in body weight with treatment compared with the controls. Dams were also necropsied at week 8 postpartum. Although pup weights were similar at birth, weight gain was lower in all test groups (data by graph only). The percent conception ranged from 81% for controls to 37% for the 50 mg/kg/day group. The incidence of dystocia was increased in all treatment groups, being 3/group at 3.125, 6.25, 25 and 50 mg/kg and 5/18 at 12.5 mg/kg/day but with no clear dose response over the 16-fold difference in doses. The percent of pups born alive at 50 mg/kg was zero (0). The percent weaned was also decreased in the treatment groups but no cause of death was established. The litters with pups with abnormalities was increased with treatment above 3.125 mg/kg, being 0/13, 0/7, 1/7, 3/16, 3/6, and 1/2, control through high dose. The historical control value was 3/313. The authors state that the difference between 12.5 mg/kg and 25 mg/kg was not statistically significant. The percent of pups with abnormalities was 0, 0, 9, 18, 13 and 14% with increasing dose compared with a historical control value of 0.1%. The most serious effect was failure of the liver to develop. Also, a number of pups had openings in the ventral abdominal wall. Cholinesterase activity was not measured. **Possible adverse effects.** Maternal NOEL < 3.125 mg/kg/day based on dystocia incidence due to atonic uterine musculature. Developmental NOEL = 3.125 mg/kg/day (litter and pup incidence of abnormalities). The study indicates maternal and developmental toxicity but has limitations in terms of interpretation due to the small group sizes and the lack of a dose response for dystocia in dams over a 16-fold range in dose. SUPPLEMENTAL. (Gee, 10/22/99).
EPA One-liner: Teratogenic NOEL = 3.1 mg/kg (LDT), teratogenic LEL = 6.3 mg/kg (lack of tail, agenesis of external genitals, failure of pubis and ischium to develop, abdominal fissures, visceral agenesis), maternal NOEL < 3.1 mg/kg (dystocia). Core grade: Supplementary.

TERATOLOGY MONKEY

Report not in CDFA file, but an unpublished 1974 gavage study is listed with EPA One-liners: Teratogenic NOEL > 20 mg/kg (HDT), maternal NOEL > 20 mg/kg. Core grade: minimum.

TERATOLOGY GUINEA PIG

169 - 099 000713 (With rebuttal and additional information in -154 and -164, 50440); "Study of Guinea Pig Teratology of SEVIN fed in the Diet versus Stomach Intubation." (report #34-81; Mellon Institute, 11/30/71) Technical grade carbaryl, 99.6% pure, in the feed at 300, 200, 100, or 0 mg/kg/day, or by oral gavage in corn oil at 200, 100, 50, or 0 mg/kg/day, on single or multiple day "windows" from day 10 through 24 (plug day = 1), with sacrifice day 34-35; MATERNAL: reduced weight gain and death at 200 mg/kg gavage; FETAL: no malformations or clear evidence of fetotoxicity in spite of maternal toxicity. GAVAGE NOEL = 200 mg/kg for developmental, 100 mg/kg for maternal; FEEDING NOEL = 300 mg/kg for developmental and maternal. UNACCEPTABLE but has useful information. J. Schreider, 5/10/85 and F. Martz, 8/7/87.

EPA One-liner for gavage: Teratogenic NOEL.200 mg/kg (HDT), maternal LEL = 200 mg/kg (decreased weight gain, mortality), fetotoxic NOEL > 200 mg/kg.

EPA One-liner for feeding: Teratogenic NOEL > 300 mg/kg (HDT), maternal NOEL > 300 mg/kg, fetotoxic NOEL > 300 mg/kg.

Core grade for both: minimum.

169 - 154, Tab C, Section IX, pg. 9: Rebuttal of #713 above. Comments are as follows:

Rebuttal: "The purpose of this study was to determine the potential teratogenicity to guinea pigs by two routes of oral administration and at maternally lethal and sublethal concentrations" [in order to assess the results of Robens (1969) showing that 300 mg/kg administered to guinea pigs by capsule caused skeletal defects albeit with 38% maternal mortality]. "Note: It is inappropriate to compare the protocol design ["teratogenic window" dosing] of this study to the routine FIFRA teratology guidelines."

Response: This was an exploratory rather than "standard" protocol study, but the various periods of organogenesis were covered by sufficient animals when the study is taken as a whole. Moreover, no fetal effects were seen in the absence of maternal toxicity at oral gavage dose levels approaching the LD_{50} established in a preliminary study for this strain of guinea pig. Considering that such treatment was more rigorous than dietary exposure, the absence of frank teratogenicity is noteworthy. Report by itself is still unacceptable, but rebuttal is accepted based on "weight of evidence." F. Martz, 5/8/87.

164, 050440; Additional data included with rebuttal for #713 above, consisting of photocopies of laboratory notebooks 1129 and 1138, as well as typed tables and reports:

Tab A: Contains the "Diet Room Record," documenting the amounts of carbaryl and feed used for diet preparation;

Tab B: Litter records consisting of the dams' sacrifice dates, the number of live or dead fetuses, resorption sites, individual fetal weights according to sex, and a description of gross anomalies if present;

Tab C: Pathology report dated 10/15/71 consisting of gross and microscopic observations of the livers and kidneys from dams receiving the top dietary or gavage dose levels as well as the respective controls;

Tab D: Pathology report dated 9/3/71 consisting of skeletal examination findings;

Tab E: Pathology report dated 12/1/71 consisting of soft tissue examination findings;

Tab F: Pathology raw data records.

(F. Martz, 5/19/87).

TERATOLOGY - GENERAL SUPPORTIVE INFORMATION

169 - 155, Tab B, no record #; Correspondence dated 5/13/85 from EPA (Douglas D. Campt) to Union Carbide (J. S. Lovell) concerning carbaryl registration standard. Among several points raised, EPA maintained its request for a repeat of the 1958 chronic dog study which was unacceptable due to major deficiencies, and extended the due date to 5/87 [Note that the new report was completed 3/18/87, received by Medical Toxicology 5/11/87, and reviewed and accepted 5/12/87]. EPA rescinded its request for a repeat dog teratology study (listed in the Registration Standard), stating that "The agency has concluded that carbaryl would not constitute a potential teratogenic hazard to humans based on the overall weight of numerous (24) teratology studies that have been conducted. We also believe that the dog is not an appropriate model to perform a teratology study and relate it to humans." **NOTE that EPA reconsidered this matter in 1986 and "concluded that it was needed" (Pesticide and Toxic Chemical News, 4/30/86).

169 - 155, 050430 An undated position paper from Drs. J.G. Wilson, A. Koestner, and C.H. Williams (recognized experts), evaluating the teratologic potential of carbaryl, with appropriate references. In their opinion, "On the basis of these animal studies, carbaryl could not be classified as a general teratogen." I agree. F. Martz, 5/87.

Regarding positive responses in dog studies at 5 mg/kg and above, they regard that "This

seemingly unique response of the beagle dog to carbaryl may in part be explained by certain metabolic peculiarities of this species with respect to this compound. The pathways for metabolism of carbaryl differ somewhat among mammalian species, but the dog stands alone in conjugating carbaryl directly, being unable to liberate 1-naphthol or to hydroxylate the parent compound (Khera, 1976). The National Institute for Occupational Safety and Health (NIOSH) in an exhaustive study of the safety of carbaryl in the workplace (Criteria for a Recommended Standard for Carbaryl, 1976), has concluded that: 'Present studies show that the metabolism of carbaryl in the dog differs from that in humans, monkeys, rats, and guinea pigs so it is unwarranted now to extrapolate from dogs to humans regarding the teratogenic potential of carbaryl.'" They agree "...with NIOSH that it would be inappropriate to use data from the dog in [developmental] safety evaluations applicable to man." F. Martz, 5/12/87.

169 - 130, 037915-24; Exact duplicate of 50430 above.

169 - 155, 050431 A 1976 EPA review/position paper from Dr. Neil Chernoff regarding reproductive and teratogenic potential of carbaryl, with appropriate references. In his opinion, "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." Based on the current weight of evidence, I agree. F. Martz, 5/12/87.

See Summary of Toxicology Data dated September 14, 1987 prepared by F. Martz with Note: The conclusion of Dr. Martz was that the data gap for teratology studies in a second species was filled with a second review by J. Parker.

GENE MUTATION

EPA One liner: There are (at least) 18 gene mutation assays. Although most had deficiencies, REAG accepted them collectively to demonstrate weakly positive response (EPA-600/6-81-001, January, 1981). These 18 studies are not on file at CDFA for independent review. In March, 1990, CDFA did not consider the data gap as filled because of the incomplete data base on file compared with that of EPA. Subsequent communication with the registrant (see 169-206) indicated these studies were not available for submission. The data gap status has been changed to filled. Gee, 7/31/90.

** 196 085660 "Mutagenicity Test on Carbaryl (Technical) in the Ames Salmonella/Microsome Reverse Mutation Assay." (Hazleton Laboratories America, Kensington, MD, HLA Study No. 10862-0-402, 9/6/89) Carbaryl technical, lot # 87191, 99.3% purity; tested with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100, triplicate plates, two trials; with and without Aroclor 1254-induced male Sprague-Dawley rat liver activation; Trial 1: 0 (DMSO), 5, 10, 50, 100, 500, 1000 ug/plate ; Trial 2: 0 (DMSO), 10, 50.

100. 500, 1000, 2000 ug/plate. **No evidence of increase in reversion rate.** Acceptable. (Gee, 2/28/90)

** 196 085658 "Mutagenicity Test on Carbaryl (Technical) in the CHO/HGPRT Forward Mutation Assay." (Hazleton Laboratories America, Kensington, MD, HLA No. 10862-0-435, 11/6/89) Carbaryl technical, lot 87191, 99.3% purity, was tested with CHO-K1-BH_4 in vitro with and without Aroclor 1254-induced rat liver activation. There was a single culture per concentration, 2 trials. Without activation, trial 1: 0 (DMSO), 0.001, 0.01, 0.03, 0.05, 0.08, 0.1, 0.15, 0.2, 0.3 (T) mg/ml; trial 2: 0 (DMSO), 0.01, 0.05, 0.1, 0.15, 0.2, 0.25 (T), 0.3 (T) mg/ml; with activation, trial 1: 0 (DMSO), 0.01, 0.05, 0.08, 0.1, 0.15, 0.2, 0.3 (T) mg/ml. In trial 2, only 1 concentration could be scored due to cytotoxicity with a new lot of S9; trial 3: 0 (DMSO), 0.001, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 (T), 0.13 (T). **No reproducible increase in forward mutations.** Acceptable. (Gee, 2/28/90)

200 090474 Revised report of 085658.

169 - 457 209660 Duplicate of 085658

CHROMOSOMAL ABERRATION

169 - 099, 027202 (with rebuttal and additional data in -154, and -166, 050442, 050956); "Comparative Study of Dietary Inclusion versus Stomach Intubation on Three-Generations of Reproduction, on Teratology and on Mutagenesis," dominant lethal portion; Mellon Institute, report #35-65, 8/31/72; technical carbaryl, 99.6% pure in feed at 200, 100, 25, 7, or 0 mg/kg/day; by gavage in corn oil at 100, 25, 7, 3, or 0 mg/kg/day; in feed containing corn oil at 100 or 0 mg/kg/day; 5 days/week (m->f); F_{2a} males withdrawn at 7 months old, mated weekly for 10 weeks:

FEEDING RESULTS: No dose-related or consistent differences, NOEL = 200 mg/kg;

FEEDING/CORN OIL RESULTS: no effects at 100 mg/kg, NOEL = 100 mg/kg;

GAVAGE RESULTS: Significant reduction in mean implants or viable fetuses at 100 mg/kg in week 8 mating only, 12.5 vs 14.0 control or 11.5 vs 14.0 control, respectively. Not regarded to be meaningful. NOEL = 100 mg/kg/day.

Originally reviewed as unacceptable without useful data (J. Schreider); now is UPGRADEABLE with additional information. F. Martz, 8/20/87.

EPA One-liner: None on file.

165 050441 Supplement to 027202.

166, 050442 & 050956; see "Reproduction Rat (Mellon report #35-65)" above for listing of entries.

169 - 179 059544 "UC 51762 - Inclusion in the Diet of Rats for Three Generations and Dominant Lethal Mutagenesis Studies." Supplemental to Record # 027202. (Carnegie-Mellon Institute, Report 42-65, 7/2/79) Positive control data with TEM given by i.p. injection to male Fischer 344 rats and mated with 1 female for each of three weekly periods. TEM caused

increased resorptions in all three weeks. Supplemental data. No change in status of # 027202. Gee, 10/3/88.

** 169 - 196 085657 "Mutagenicity Test on Carbaryl Technical: In an in vitro Cytogenetic

Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells." (Hazleton Laboratories America, Kensington, MD, HLA Study no. 10862-0-437, 8/31/89) Carbaryl technical, Lot # 87191, 99.3%; tested with CHO-WBL cells in vitro for chromosomal aberrations; without S9, at 0 (negative and solvent), 7.5, 10, 25, 50 or 75 ug/ml, 17.5 hours incubation and 20-hour harvest, duplicate cultures; with Aroclor 1254-induced Sprague-Dawley rat liver S9 activation at 0 (negative and solvent), 150, 200, 250 or 300 ug/ml, duplicate cultures, 2 hour incubation and harvest at 20 and at 30 hours; harvest times based on a preliminary study with BrdUrd staining for determination of cell cycles in 27.5 hours total; no increase in aberrations without activation; **possible adverse effect** with activation - increase in aberrations/cell, % cells with aberrations and % cells with >1 aberration at both harvest times. Acceptable. (Gee, 2/27/90)

EPA One liner: EPA considers the following studies acceptable for filling the chromosome mutation data gap: Can. J. Genet. Cytol., 8:481-501, 1966.; Cytologia, 30:175-181, 1965; Cytologia, 33:334-337, 1968; Flora (Jena), 160:443-439, 1971; Archiv za Poljoprivedne Naake, 25:128-138, 1973. The studies cited by EPA above need to be submitted for independent review. Gee, 3/90.

** 169 - 0458 209661 " Carbaryl: Induction of micronuclei in the bone marrow of treated mice." (Marshall, R., Corning Hazleton (Europe), Study number 198/89-1052, March 13, 1996) CD-1 mice were treated with carbaryl (lot OP 9450293, 99.9%) in 0.5% carboxymethylcellulose at doses of 0, 50, 100 or 200 mg/kg/day, for two consecutive days, with 5/sex/dose sacrificed after a further 24 or 48 hours. Cyclophosphamide was used as the positive control and was functional. At 200 mg/kg, animals showed lethargy which lasted about 2 hours after the first dose with eye closure in 3 females, eye secretions in 1. Weight loss was seen in 2 males and 10 females at the high dose. For each animal, 2000 polychromatic erythrocytes were scored for micronuclei and the PCE/NCE reported. There was no induction of micronuclei by carbaryl in this study. ACCEPTABLE with no adverse effect. (Gee, 3/2/04).

DNA DAMAGE/REPAIR AND OTHER

EPA One liner: There are 3 DNA repair assays (Mutation Research, 42:161-174, 1977; Mutation Research, 38:293-302, 1976; Mutation Research, 22:121-126, 1974) which collectively fill the data gap. These studies are not on file at CDFA and need to be submitted. Gee, 3/5/90.

** 196 085659 "Mutagenicity Test on Carbaryl Technical in the in vitro Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay." (Hazleton Laboratories America, Kensington, MD, HLA Study No. 10862-0-447, 11/22/89) Carbaryl technical, lot 87191, 99.3% purity; tested with primary rat hepatocytes from male Fischer 344 rats, two trials; trial 1: 0 (DMSO), 0.5, 1.0, 2.5, 5, 10 or 25 ug/ml; trial 2: 0 (DMSO), 5.0, 7.51, 10, 15, 20 or 25 ug/ml; scored 150 cells per concentration from triplicate coverslips; **no evidence of unscheduled DNA synthesis.** Acceptable. (Gee, 2/28/90)

** 169 - 0456, 0466 209659, 212614 " Investigation of the potential for protein- and DNA-binding of carbaryl." (Sagelsdorff, P., Ciba-Geigy, Basel, CB93/52, R013980, April 28, 1994) Covalent binding of ^{14}C-carbaryl to chromatin protein and to DNA was determined using male CD-1 mice. There were 5 groups of 4 - 6 per group treated as follows: Group 1 received one dose of 75 mg/kg of radioactive carbaryl by gavage; group 2 were fed 8000 ppm for 13 days, then given radioactive carbaryl in a single dose at 75 mg/kg body weight; group 3 were untreated and used for controls of the extraction procedures; group 4 were fed 8000 ppm for 14 days and group 5 were untreated. Groups 4 and 5 were not processed [see 169-455, 209658].

Urinary excretion was measured for a single animal from groups 1 and 2 over 24 hours and found to be 33 and 31%, respectively, of the administered dose. Fecal excretion was not measured. Livers from 2 animals of the same group were pooled for processing. Livers were homogenized, chromatin precipitated, deproteinated and DNA further purified on hydroxylapatite, dialyzed and precipitated with ethanol. Chromatin protein was precipitated with acetone and dissolved in 1% SDS several times. Radioactivity was determined by LSC. Binding was determined as a function of mg of protein and of DNA. The pmol/mg binding to protein ranged from 7 to 11, with no difference between groups 1 and 2. For DNA, binding (dpm/mg) was <5.99 with an 80% counting efficiency and a limit of detection of 2.7 cpm over background. The Covalent Binding Index (CBI) was calculated to be <0.1 as a maximum DNA-binding ability. This result gives no indication for a genotoxic potential for carbaryl mediated by DNA binding. The CBIs for strong hepatocarcinogens (such as aflatoxin B1) are magnitudes higher. Record 212614, 169-0466, contains the publication detailing the methods used in the study. ACCEPTABLE with no indication of DNA binding. (Gee, 7/20/04)

169 - 0466 212614 "The relevance of covalent binding to mouse liver DNA and to the carcinogenic action of hexachlorocyclohexane isomers." (Sagelsdorff, P., W. K. Lutz and C. Schlatter, publ. in *Carcinogenesis* 4:1267 - 1273 (1983). This submission was related to the one above, record 209659, giving details of the methods used in the isolation and quantitation of DNA and protein for binding analysis. (Gee, 7/20/04)

169 – 399 177756 D. Bigot "Validation on Transgenic Mice – p53 Knockout Mice – to Predict Rodent Carcinogenicity." (Rhone-Poulenc Agro, Study SA 97040, 11/10/99) Male mice, C57Bl/6 Tac-[KO]Trp53N5-T, heterozygous for p53 tumor suppressor gene, were compared with wild type male mice for response to the urethane, a genotoxic compound known to induce vascular tumors in lifetime studies in mice, and to d-limonene, not carcinogenic in mice. There were 20 mice per group, given 0, 1, 10 or 100 mg/kg/day of urethane by gavage for at least 180 days or d-limonene at 250 mg/kg/day. Wild type mice were given vehicle only. Body weights and food consumption were recorded as were clinical signs. At necropsy, all major organs were examined, selected organs weighed, and tissues prepared for histopathological examination. At 100 mg/kg urethane, only 3 animals survived to termination with 2 dying at 10 mg/kg. A total of 18/20 mice at 100 mg/kg urethane had vascular neoplasms, predominantly in the liver, at 181 – 184 days. At 10 mg/kg, 1/20 had a vascular tumor and none were seen at 1 or in the vehicle control group. The results supported urethane as a model for vascular tumors in p53 knockout mice induced by a genotoxic carcinogen. D-Limonene exposure resulted in hyperplasia of the non-glandular stomach but was considered negative for tumor induction. This study was submitted as support for record no. 177755 with carbaryl. No worksheet. (Gee, 3/28/06)

169 – 398 177755 F. Chuzel "Carbaryl 6-Month Carcinogenicity Study in p53 Knockout Mice by Dietary Administration." (Rhone-Poulenc Agro, Study SA 98155, 7/8/99) Carbaryl (99% purity) was fed in the diet to groups of 20 male mice for at least 180 days. Mice were C57Bl/6 Tac fBR-[KO]p53N4, heterozygous for the p53 tumor suppressor gene. Doses were 0, 10, 30, 100, 300, 1000 or 4000 ppm (mean achieved doses were 0, 1.76, 5.21, 17.5, 51.6, 164.5 and 716.6 mg/kg/day). The purpose was to better understand tumors seen in the earlier study, record 123769, N. Hamada, HWA 656-138, 5/20/93 [see above under mouse oncogenicity]. This strain of knockout mice has been shown to respond to genotoxic carcinogens in a shorter time frame than in a usual bioassay, forming tumors in the first six months of life, before the spontaneous incidence increases. See record 177756 above. Body weight, food consumption and clinical signs were recorded. Selected organs were weighed and tissues prepared for histopathological 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 incidences were: 0/20. 0/20, 0/20, 11/20, 20/20, 20/20 and 20/20, control through high dose. 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 = 30 ppm (5.2 mg/kg). There was no treatment-related evidence of neoplastic or preneoplastic changes in vascular tissue or any organs examined. Several spontaneous neoplasms were found with none, however, present at 4000 ppm. This study did not demonstrate a genotoxic potential for carbaryl. Supplemental study. No worksheet. (Gee, 3/29/06)

NEUROTOXICITY, HEN

169 - 134 037928 (with rebuttal and additional information in -154 and -156, 50432) "Comparison of the Demyelination Potential of SEVIN and Triorthocresyl Phosphate in Chickens, with Observations on the effects in Liver, Kidney, and gastrocnemius Muscle Tissue." (Mellon Institute, report #21-87, 9/15/58) No brain, spinal cord or sciatic nerve effects at 3 g/kg, subcutaneously, but results were inconclusive because of weak TOCP effect also at 3 g/kg. F. Martz, 5/4 and 8/7/87 (no worksheet).
EPA One-liner: Negative at 2000 mg/kg (approximate LD_{50}). Core grade: minimum.

154, Tab C, Section I, pg. 1, and 169-156, 50432, rebuttal to 169-023, no record #; Rebuttal not necessary because study is not required, inasmuch as carbamates have no documented neurotoxic potential such as that exhibited by organophosphates. F. Martz, 8/7/87 (no worksheet).

NEUROTOXICITY, DEVELOPMENTAL, RAT

** 169 - 384 166126 "A developmental neurotoxicity study of orally administered carbaryl, technical grade, in the rat." (K. Robinson and B. Broxup, ClinTrials BioResearch Ltd., Quebec, Project 97391, 9/23/97). Sprague-Dawley Crl:CD(SD)BR rats were treated with carbaryl, 99.1% purity, by oral gavage at doses of 0 (aqueous 0.5% carboxymethylcellulose/0.1% Tween 80), 0.1, 1.0 or 10 mg/kg/day, day 6 of gestation through day 10 post partum. There were 26 per group for the developmental neurotoxicity phase and 6 per group for cholinesterase determinations. Both F0 adults and F1 generation were examined by a "modified" Functional Observation Battery. Additional parameters for pups were also recorded including motor activity, brain measurements, development (tooth eruption, eye opening, vaginal opening, preputial separation) and gross and microscopic pathology. Effects on F0 dams at 10 mg/kg/day included autonomic effects and tremors seen during the treatment period, inhibition of RBC, whole blood and brain cholinesterase at 10 mg/kg. Maternal NOEL = 1 mg/kg/day. In the F1 generation, there were no effects on FOB, motor activity, startle response, avoidance, water maze times, body weight, brain morphometric measurements, or pathology. Developmental neurotoxic NOEL = 10 mg/kg/day. No positive control data were included or cited. Unacceptable but upgradeable with information concerning appropriate positive control studies. No adverse effects. (Gee, 2/16/99)

Note: A considerable body of positive control studies conducted at BioResearch has been submitted. See below. These studies upgrade the developmental neurotoxicity study to ACCEPTABLE status. No new worksheet. (Gee, 7/27/04)

169 - 391 170648 Supplement to 169-384 166126 Supplement date of June 1, 1999. Authors were K. Robinson and B. Broxup. At the request of US EPA for additional morphometric measurements to assist in the interpretation of the occasional statistically significant differences in specific areas of the brain between the control and high-dose pups and adults, the measurements were repeated. Evaluation of the mid- and low-dose groups was stated as not possible due to the lack of appropriate control tissues with the passage of time. The reevaluation confirmed some of the original findings. These were, again, discounted as treatment-related by the authors based on such criteria as unilateral finding, not seen in both pups and adults, found in one sex only, and not statistically significant based on the adjusted P-value. This submission did not address the positive control data requested by DPR for an upgrade of the study. SUPPLEMENTAL. (Gee, 9/10/99).

NEUROTOXICITY, RAT

169 – 396 177090 "An experimental functional observational battery validation study with carbaryl in Wistar rats" (Wahle, M. S., Bayer Corporation, Stilwell, KS, Report 109406, 7/26/00) The purpose of the study was to validate the procedures of the Functional Observational Battery using untreated animals and animals exposed to a substance with known effects, carbaryl, to serve as positive control data under FIFRA guidelines for neurotoxicity studies. Four technicians were involved. Procedure: Male Wistar Hanover rats (total of 40) were subjected to FOB observations before treatment, 10 animals per technician. Six per group were then given 0 (vehicle: 5% (v/v) ethanol and 5% (v/v) Cremophor EL), 15 or 30 mg/kg carbaryl (99%) by intraperitoneal injection, single dose. At 20 to 90 minutes post-dosing, animals were subjected to an FOB and observed by the four technicians. Compound-related effects at 15 mg/kg included a variety of autonomic signs, alterations in CNS excitability, neuromuscular effects, decreased sensorimotor responses and alterations in activity. The effects at 30 mg/kg were increased in incidence and severity. The observations of each technician were reported and compared. With a few exceptions, there was good agreement among the observers. The study supported the validity and sensitivity of the procedures and training of personnel. Supplemental study. (Gee, 9/20/2000)

** 169 - 341 142602 " An acute study of the potential effects of a single orally administered dose of carbaryl, technical grade, on behavior and neuromorphology in rats." (Brooks, W., K. Robinson and B. Broxup, Bio-Research Laboratories, Quebec, Project 97389, October 24, 1995) Carbaryl (lot 201085006, 99.1%) was given in a single oral dose by gavage at nominal doses of 0 (Aqueous 0.5% carboxymethylcellulose/0.1% Tween 80), 10, 50 or 125 mg/kg to twelve Sprague-Dawley (Crl:CD®(SD)BR) rats per sex per dose. Dosing was done over 3 days with 4/sex/group dosed each day. Observations included a FOB, motor activity, and brain weight and measurements over a 14 day period following dosing. The observations were made predosing, on day 0 at 0.5 hours for the FOB and 50 to 90 minutes after dosing for motor activity. These observations were repeated on days 7 and 14. On day 15, six per sex per dose were perfused and histopathology performed on the control and high dose animals. NOEL for clinical signs and FOB = 10 mg/kg. There was, however, a statistically significant decrease in motor activity on day 0 at 10 mg/kg, being 177.2 ± 59 versus 221.7 ± 51.3 for males and 314.3 ± 101.6 versus 393.8 ± 127.6 for females. Motor activity was measured over a total of 60 minutes. There was also a statistically significant lower body temperature in females at 10

mg/kg (37.46*** versus 38.52 in controls) but this was discounted as not being a reflection of neurotoxicity and as comparable to the historical control range of 37.6 to 38.8°C. Males at 10 mg/kg had temperatures comparable to controls, day 0. Among the observations on day 0 were tremors, lower body temperature, decreased motor activity with the effects being greater at 125 mg/kg than at 50 mg/kg. All animals were comparable to controls by day 7. Positive control data for FOB and neuropathology were submitted on a CD. Acceptable. (Gee, 7/26/04)

** 169 - 0459, 0465 209662, 212613 " A 13 week study of the potential effects of orally administered carbaryl, technical grade, on behavior, neurochemistry and neuromorphology in rats." (Robinson, K. and B. Broxup, Bio-Research Laboratories, Ltd., Quebec, Laboratory ID project 97390, R014070, September 24, 1996) Sprague-Dawley Crl:CD®(SD)BR male and female rats were given technical grade carbaryl (99.1%) by oral gavage for 90 days at 0 (0.5% (w/v) carboxy-methylcellulose/0.1% Tween 80), 1, 10 or 30 mg/kg/day nominal doses. A total of 27/sex received each dose. Twelve per sex per dose were used for the behavior evaluations prestudy and week 4, 8 and 13. Groups of 5/sex/dose were used for cholinesterase prestudy and each of weeks 4, 8 and 13 with brain cholinesterase also being determined. Cholinesterase activity was reported for RBC, whole blood, plasma, left hemisphere and selected regions of the brain. Cholinesterase activity was lower in most samples at 10 and 30 mg/kg/day for blood and brain. The major FOB observations at 10 and 30 mg/kg were decreased pupil size, tremors of the head/body/limbs and reduced rearing of females at 4 and 8 weeks. At 30 mg/kg/day, additional observations included increased salivation. Decreased body temperature was noted in females at 10 and 30 and in males at 30 mg/kg/day. Decreases in motor activity were noted on occasion at 30 mg/kg/day. Some clinical findings were of lower incidence at week 13 than at earlier times. Six/sex/group were perfused for neuropathology, including brain measurements. There were no treatment-related histopathological findings in the nervous system. NOEL = 1 mg/kg/day (tremors, decreased pupil size, reduced activity and cholinesterase activity). Record 212613 contains a brief description of the method used for cholinesterase measurements and the individual data for blood parameters, specifically hematocrit, used in RBC cholinesterase calculations. ACCEPTABLE. Positive control data were submitted on a CD. (Gee, 7/26/04)

POSITIVE CONTROL STUDIES submitted on a compact disc. Selected pages of each study have been printed and are on file in the Medical Toxicology Branch under CC 105, carbaryl.

Project 29537: Compact disc. "An acute neurotoxicity study of the effects of orally administered DDT and trimethyltin chloride in rats." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 11/4/94) Male rats were given doses of 0, 112.5 mg/kg DDT or 9 mg/kg trimethyltin chloride, single oral dose. They were examined prestudy, day 0 (following dosing) and days 7 and 14 for FOB, motor activity and neuropathology following perfusion (controls and TMT only). All essential parameters of home cage, handling, arena, FOB, grip strength, foot splay and pathology were examined and reported, including all individual data. The expected responses were noted. DDT caused tremors, decrease in rearing, were hyper-reactive when handled, diarrhea, increased hindlimb splay and decreased activity counts on day 0 compared with controls. For TMT, with the exception of a slightly lower temperature, all values were similar to controls on day 0. On day 7, however, TMT caused increased rearing and locomotor activity in the arena and 2/12 had "shakes when handled" and 2/12 were "difficult to handle." On day 14, TMT animals had increased forelimb grip strength and increased hindlimb splay (not seen on day 7). On both days 7 and 14, TMT caused increased activity counts compared with controls and DDT exposed rats. The major finding with TMT was the expected neuropathology

of the central and peripheral nervous system in perfused animals, day 15. No gross changes were recorded but microscopic findings included necrosis of neurons, occasionally associated with neuronophagia and gliosis, in sections of the brain. In the peripheral nervous system, slight to moderate changes were seen including axonal degeneration and swelling, myelin splitting/bubbling, Schwann cell hypertrophy/hyperplasia and interstitial edema. This report is an acceptable study to support the acute and subchronic neurotoxicity studies with carbaryl. No worksheet. (Gee, 7/21/04) Duplicate of 52093-080, record 156318.

Project 29538: CD "A subchronic neurotoxicity study of the effects of orally administered acrylamide in rats." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 11/4/94) Male Sprague-Dawley rats (12/group) were given either vehicle (water) or 40 mg/kg/day of acrylamide by oral gavage. Doses were repeated for 12 days. Animals were examined prestudy, days 7 (predosing) and 14. Parameters included the FOB, motor activity and neuropathology. Animals were sacrificed on day 15 with 6/group being chosen randomly for perfusion and subsequent pathological exam. One male given acrylamide died before completion of the study. Mean body weights were significantly lower with acrylamide throughout the study, being 280.8*** at day 14 versus 361.0 in controls. The major clinical observations with acrylamide were hypersensitive/aggressive behavior when handled (10/12) and abnormal gait (8/12). During the FOB on day 7, significant affects included hypotonic gait (8/12), decreased arousal (5/12), flaccid abdominal tone (6/12) and increased hindlimb splay. On day 14, all neuromuscular parameters were affected (flaccid muscle tone, ataxic gait, reduced fore and hindlimb grip, increased hindlimb splay, and decreased locomotor activity, arousal and rearing incidents in the arena, others). Motor activity was also reduced on both days 7 and 14. Neuropathological examination showed damage (e.g axonal degeneration) to the peripheral nerves, ganglia and roots and lesions in the CNS (degeneration/necrosis of purkinje cells of the cerebellum). This report is an acceptable study to support the acute and subchronic neurotoxicity studies with carbaryl. No worksheet. (Gee, 7/21/04). Duplicate of 52093 - 081, record 156319.

Project 29546: CD. "An acute neurotoxicity study of the effects of orally administered carbaryl and triadimefon in rats." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 11/4/94) Male Sprague-Dawley rats (12/group) were given a single oral dose by gavage of 0 (0.5% carboxymethylcellulose/0.1% Tween 80 (w/v)), triadimefon at 100 mg/kg or carbaryl (lot 81-25B, 97%) at 40 mg/kg. Animals were evaluated for motor activity and FOB prestudy, day 0 (day of treatment, 1 - 1 1/2 hours post dosing), and days 7 and 14. All animals were discarded on day 15. No necropsy/histopathology was performed. Body weights were comparable in all groups. Triadimefon treatment resulted in significant increases in locomotor activity, rearing and arousal in the arena on day 0. With carbaryl, day 0, there were significant increases in tremors (9/12), salivation (8/12), and pupil constriction (pinpoint, 8/12), decreased rearing in the arena (1.8 versus 8.9 in control), overall gait incapacity (9/12), decreased locomotor activity (7/12) and depressed arousal (8/12), others. Grip strength and hindlimb splay were comparable to controls. There was a significant decrease in motor activity, day 0, with carbaryl, being overall (60 minutes) 60.7*** versus 235.1 in controls. Triadimefon activity was increased to 544.8, as statistically significant. By day 7, all groups were essentially comparable. The study gave the anticipated results with these two compounds. This report is an acceptable study to support the acute and subchronic neurotoxicity studies with carbaryl. No worksheet. (Gee, 7/21/04) Duplicate of 52093 - 083, record 156321.

Project 97104: CD. "A benchmark study of the acute toxicity of DDT (1,1-bis[p-chlorophenyl]-2,2,2-trichloroethane) in the albino rat." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 6/16/92) Pairs of male Sprague-Dawley rats were given a single oral dose of DDT by

gavage at doses of 0 (corn oil), 50, 75, 112.5, 168.8, 253.1 or 379.7 mg/kg. They were observed for 1 week. At 379.7 and 253.1, both animals died within 6 and 21 hours, respectively. All rats showed head, body and/or limb tremors commencing 4 hours after dosing. Clonic-type convulsions were seen in 1 animal each at 112.5 and 379.7mg/kg, just prior to death. Other signs included hyper-reactivity to sound, piloerection and nasal/ocular discharge. The "benchmark" dose (highest non-lethal dose) was 168.8 mg/kg. This study is supplemental as a positive control study. No worksheet. (Gee, 7/22/04)

Project 97107: CD. "A benchmark study of the acute toxicity of carbaryl in the albino rat." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 6/16/92) Pairs of male Sprague-Dawley rats were given carbaryl (lot 56-98A, P5-84, 98%) as a single dose of 0 (aqueous 0.5% (w/v) carboxymethylcellulose/0.1% Tween 80), 125, 250, 500 or 1000 mg/kg by oral gavage. Animals were observed for 1 week. At 1000 mg/kg, both animals died on the day of dosing and 1 animal at 500 mg/kg was dead 2 days after dosing. All treated rats showed tremors of the head, body and/or limbs beginning about 10 minutes after dosing. Autonomic signs (salivation, lacrimation, urinary staining) were seen within 6 hours. Other signs seen on the day of treatment included muzzle staining, abnormal respiratory rate/abnormal sounds/gasping, exophthalmus and flattened body position. All treated groups lost weight from day 0 to day 1. Generally, findings were no longer noted 1 to 2 days following treatment. The "benchmark" dose (highest non-lethal dose) was 250 mg/kg. This study is supplemental as a positive control study. No worksheet. (Gee, 7/22/04)

Project 97108: CD. "A time of peak effect study of an acute dose of carbaryl in the albino rat." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 10/22/92) Pairs of Sprague-Dawley male rats were given a single dose of carbaryl (lot No. 56-98A, 98%) at 0 (aqueous 0.5% carboxymethylcellulose/0.1% Tween 80), 25, 80 or 250 mg/kg. Animals were assessed with an abbreviated FOB at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hours post-dosing. Animals were observed for a total of 7 days, including body weight measurements. Rats given 250 mg/kg showed decreased arousal and locomotor activity with the largest effect at 1 - 1.5 hours. Other findings included incapacitated gait, tremors, salivation, lacrimation (beginning at 4 hours), urinary staining (beginning at 4 hours) and reduced respiration. At 80 mg/kg, locomotor activity was decreased with the greatest effect from 0.5 to 3 hours. Arousal was reduced the most from 0.5 - 1 hour. Other findings at 80 mg/kg included incapacitated gait, tremors, salivation and reduced respiration. At 25 mg/kg, animals were comparable to controls. Body weight losses were seen at 80 and 250 mg/kg. NOEL = 25 mg/kg. The estimated peak time of effect was 0.5 to 1.5 hours post-dosing. Supportive information for neurotoxicity studies. No worksheet. (Gee, 7/22/04)

Project 97109: CD "An acute study of the potential effects of orally administered carbaryl on behavior in rats." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 11/3/92) Groups of 12 male Sprague-Dawley rats were given a single dose of 0 (aqueous 0.5% carboxymethylcellulose/0.1% Tween 80), 12.5, 40 or 125 mg/kg carbaryl (lot 56-98A, P5-84, 98%) by oral gavage. No analysis of dosing material. Animals were evaluated using a FOB and motor activity at pretest and days 0 (day of treatment), 1, 7 and 14. Histopathology was limited to sections of the brain and abnormal tissues. No animals died or were sacrificed. Body weight at 125 mg/kg was significantly lower on days 1 (9.5%) and 7 (6%), resulting from a mean loss of 27 g from day 0 to day 1. For the FOB at 125 mg/kg, day of treatment, there were significant increases in tremors (12/12 vs. 0), gait incapacity (11/12 vs. 0), and autonomic signs (salivation, miosis). They showed reduced locomotor activity (12/12), arousal (11/12), decreased defecation, abnormal responses to sensory tests and others. At 40 mg/kg, animals showed increases in tremors (11/12), salivation and decreased locomotor activity (11/12 v 2/12

in control), arousal (11/12), toe/tail pinch and decreased defecation. At 12.5 mg/kg, defecation was also reduced on day 0. By day 1, all groups were similar. Forelimb and hindlimb grip strength was reduced at 125 mg/kg, day 0, and hindlimb at 40 mg/kg. Foot splay was significantly increased for both groups. Body temperature was lower for all groups, being 38.0, 37.3*, 34.9** and 34.3** for control through increasing dose. Group mean total activity counts, day 0, were lower at 40 and 125, being 209, 207, 43.3*** and 23.7***, control through increasing dose. There were no significant differences on days 7 and 14. There were no apparent treatment-related histopathology findings in the brain. NOEL = 12.5 mg/kg, based on FOB findings on the day of treatment. This report supports the definitive acute and subchronic neurotoxicity studies with carbaryl. No worksheet. (Gee, 7/22/04)

Project 97110: CD. "A benchmark study of the acute toxicity of trimethyltin chloride in the albino rat." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 6/26/92) Pairs of male Sprague-Dawley rats were given a single oral dose by gavage at 0 (corn oil), 5.3, 8, 12, 18 or 27 mg/kg. They were observed for 7 days. On day 2, they were subjected to a limited FOB including arena/handling. The brain from each surviving animal was retained and examined (6 coronal sections) using hematoxylin and eosin and Kluver Barrera stains. All animals at 12 mg/kg and above died before termination of the study but lived long enough to yield data. Both males at 12 and at 27 and one at 18 displayed tremors by day 3, lasting until death. Aggressive behavior was noted at 12 mg/kg on day 3 of handling. The FOB on day 2 showed tremors, decreased locomotion, impaired gait at 27 mg/kg. By day 7, rats given 12 mg/kg showed a large decrease in body weight (-103 g). Histopathology of the brain showed neuronal necrosis of the limbic system (hippocampus, pyriform cortex, entorhinal cortex) at all TMT doses with the severity being greater at 12 mg/kg and above. The "benchmark" dose (highest non-lethal and adequate to cause neuropathology) was 8 mg/kg, single dose. This study provides neuropathology positive control data for the neurotoxicity studies with carbaryl. No worksheet. (Gee, 7/22/04)

Project 97111: CD: "A time of peak effect study of an acute dose of trimethyltin chloride in the albino rat." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 10/22/92) Pairs of Sprague-Dawley male rats were given single doses of trimethyltin chloride by oral gavage using doses of 0 (corn oil), 4, 6 or 9 mg/kg. Animals were given an abbreviated FOB predosing and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hours post dosing. The FOB included locomotor activity and arena/handling observations. After 14 days, all animals were sacrificed and the brain retained for histopathology. Six coronal levels were stained with hematoxylin and eosin or Kluver Barrera stains. No clear time of peak effect was noted but arousal at 9 mg/kg was slightly increased at all times and locomotor activity was increased. According to the author, this occurred between 5 and 7 hours post dosing. Histopathology of the brain showed neuronal necrosis of the limbic system (ammon's horn, pyriform cortex and entorhinal cortex) in all doses, with severity being greatest at 9 mg/kg. At 4 mg/kg, 1 of 2 showed slight pathology. This study provides neuropathology positive control data for the neurotoxicity studies with carbaryl. No worksheet. (Gee, 7/23/04)

Project 97112: CD. "A benchmark study of the acute toxicity of triadimefon in the albino rat." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 6/16/92) Pairs of male Sprague-Dawley rats were given triadimefon (lot 46-114A, 99%) in a single oral dose by gavage of 0 (aqueous 0.5% carboxymethylcellulose/0.1% Tween 80), 150, 300, 600 or 1200 mg/kg. Animals were followed for 7 days, being observed for clinical signs (0.5, 1, 2, 4 and 6 hours post dosing) and body weight. After 7 days, animals were terminated without necropsy. Both animals at 1200 mg/kg and 1 at 600 mg/kg were terminated on day 1 due to poor condition. All treated animals showed hyperactivity, starting within 0.75 hours of treatment, and "sniffing",

beginning at 4 hours. Ataxia was seen at 300 mg/kg and higher, bizarre behavior (including self mutilation) at 600 mg/kg. Other effects were also seen. All treated animals had weight loss from day 0 to day 1, with overall weight gain over 7 days being lower. No NOEL was determined. The "benchmark" dose, defined as the highest non-lethal dose, was 300 mg/kg. This study is supplemental to the positive control data. No worksheet. (Gee, 7/23/04)

Project 97113: CD. "A time of peak effect study of an acute dose of triadimefon in the albino rat." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 10/22/92) Pairs of male Sprague-Dawley rats were given a single oral dose of triadimefon by gavage at doses of 0 (aqueous 0.5% (w/v) carboxymethylcellulose/0.1% Tween 80), 30, 100 or 300 mg/kg. Animals were examined at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8 and 24 hours post dosing using an abbreviated FOB (locomotor activity, arena/handling). After 7 days, animals were terminated and discarded without necropsy. Locomotor activity was increased from hours 1 - 8 post dosing with the largest difference being from 1.5 - 3 hours. Arousal was increased at 100 mg/kg, hours1-5, and at 300 mg/kg, hours 1 - 8. There was marked weight loss days 0 - 1 at 300 mg/kg (35.5 g), resulting in lower body weight day 7. The overall peak time of effect was 1.5 - 3 hours post-dosing. This study is supplemental to the positive control data. No worksheet. (Gee, 7/23/04).

Project 97132: CD. "An acute study of the potential effects of orally administered triadimefon on behavior in rats." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 276 pages, 11/19/92) Groups of 12 male Sprague-Dawley rats were given a single oral dose of triadimefon (lot 46-114A, 99%) by gavage at 0 (aqueous 0.5% (w/v) carboxymethyl-cellulose/0.1% Tween 80), 30, 100 or 300 mg/kg. Animals were observed for 14 days, being assessed for motor activity and full FOB prestudy, day 0 (estimated time of peak activity - not stated) and days 1, 7 and 14. On day 15, animals were terminated and subjected to necropsy. The brain was retained from 6/group for possible future analysis. Motor activity was significantly increased in all groups. Rearing was increased at 300 mg/kg and arousal was increased at 100 and 300 mg/kg. There was no effect on temperature, grip strength or splay. Also, there were no gross necropsy findings. This study is supplemental to the positive control data. No worksheet. (Gee, 7/26/04)

Project 97134: CD. "An acute study of the potential effects of orally administered DDT [1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane] on behavior in rats." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 303 pages, 11/20/92) Groups of 12 male Sprague-Dawley rats were given doses of 0 (corn oil), 11.3, 35 or 112.5 mg/kg in a single dose by gavage. The animals were evaluated by FOB and motor activity prestudy and on days 0 (at estimated time of peak effect - not stated), 1, 7 and 14. On day 15, 6/group were selected for perfusion and neuropathological examination. The rest were examined for necropsy. There was no effect on body weight. On day 0, increase in tremors, difficulty of removal from home cage, muzzle staining, reduced tail pinch and delays for positional passivity test were noted at 112.5 mg/kg. Also, there was a reduction in arousal, pinna reflex, increased urination, diarrhea, and hyperreactivity to sound/increased auditory startle. On day 1, at 112.5 mg/kg there was a significant increase in locomotor activity, arousal and rearing. Hindlimb grip strength was lower at 112.5 mg/kg and body temperature was higher on day 0. No abnormal gross or neuropathology findings were reported. This report supports the definitive acute and subchronic neurotoxicity studies with carbaryl. No worksheet. (Gee, 7/26/04)

Project 97135: CD. "A subacute study of the potential effects of orally administered acrylamide on behavior and neuromorphology in rats." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 225 pages, 11/13/92) Groups of 12 male Sprague-Dawley rats were given 10 doses over 14 days at 0 (water), 4, 13 or 40 mg/kg/day by gavage. Animals were

evaluated using a FOB and motor activity. On day 15, 6 per group were selected for perfusion and control and high dose animals were evaluated for neuropathology. Brains were saved from the other 6/group for possible future evaluation. Body weight at 40 mg/kg was significantly lower (-12.9%) on day 14. The FOB on day 14 showed a significant increase in the incidence of ataxic gait, incapacitation and flaccid body/abdominal tone (10/12) at 40 mg/kg. There was also a reduction in fore and hindlimb grip strength (546.7 versus 735 g in controls), increase in hindlimb splay (11.1* versus 8.5 cm in controls), and a decrease in total activity counts (96.9 versus 238.7 in controls). No gross pathological findings but there was a decrease in brain width. Neuropathology also indicated degeneration of peripheral nerves, changes in cell nuclei and cell body cytoplasm (granularity) of spinal root ganglia at the high dose. Necrosis of the purkinje cells and vacuolation of the neurophil of the cerebellum were also noted at 40 mg/kg/day. This study provides neuropathology positive control data for the neurotoxicity studies with carbaryl. No worksheet. (Gee, 7/26/04).

Project 97136: CD. "An acute study of the potential effects of orally administered trimethyltin chloride on behavior, neuromorphology and neurochemistry in rats." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 302 pages, 11/20/92) Groups of 12 male Sprague-Dawley rats were given a single dose of trimethyltin chloride at 0 (corn oil), 4, 6 or 9 mg/kg and followed for 2 weeks. Animals were evaluated with a FOB and for motor activity prestudy, day 0 (at estimated time of peak effect - not stated) and days 7 and 14. On day 15, 6 per group were sacrificed and given a necropsy with the brain removed for GFAP (glial fibrillary acidic protein) analysis in six regions of the brain and the other 6 in control and high dose were perfused for neuropathological evaluation and stained with hematoxylin and eosin, Kluver-Barrera and Holmes stains for light microscopy. Body weight at 9 mg/kg was lower on day 7. In the FOB, at 9 mg/kg, there was an increase in the incidence of rats lying on side/curled up in the home cage on day 0 (6/12). By day 7, locomotor activity, toe pinch response and vocalization were increased at 9 mg/kg. By day 14, locomotor activity remained increased. There was no difference in grip strength, foot splay or temperature. Motor activity was increased on days 7 (377 versus 202 in control group) and 14 (418 versus 216 in controls) at 9 mg/kg. There were no gross pathological findings related to treatment but neuropathological exam found neuronal necrosis and astrocytosis of the hippocampus and pyriform cortex of the brain. Changes in the peripheral nervous system, primarily in the sciatic and tibial nerves, lumbar dorsal root and lumbar dorsal root ganglion such as myelin splitting and bubbling were seen. There were significant increases in GFAP at the high dose for the cerebral cortex and striatum regions. This study provides neuropathology positive control data for the neurotoxicity studies with carbaryl. No worksheet. (Gee, 7/26/04).

Project 97162: CD. "An inter-observer reliability (IOR) study for qualitative functional observational battery assessments in rats." (Beyrouty, P., Bio-Research Laboratories, Quebec, Canada, 88 pages, 11/18/92) Pairs of male Sprague-Dawley rats were treated with a single dose by gavage of DDT (100 mg/kg), carbaryl (100 mg/kg) or acrylamide (40 mg/kg). The animals were assessed by a group of 5 observers to assess major neurotoxic endpoints. All observers detected the expected findings within 1 grade for ranked data. The five observers were identified by a letter. The conclusion was that the observations would be suitable for studies and reliable if more than one observer was used in a given study. No worksheet. (Gee, 7/26/04).

See also 52093 - 082, record 156320, for a similar study of inter-observer reliability, 11/4/94, Bio-Research Laboratories project 29540, submitted with cyclanilide. Reviewed by Aldous, 10/8/97.

Project 97163: CD. "An inter-observer reliability (IOR) study for grip strength and hindlimb splay measurements in rats." (Beyrouty, P., Bio-research Laboratories, Quebec, Canada, 51 pages, 11/16/92) Ten male Sprague-Dawley rats were assigned to each of 5 observers. All animals had been tested on day -6 by a single experimenter. No differences were noted among the 5 observers for forelimb strength or hindlimb splay. There was, however, a difference for hindlimb strength. Following additional training, the results were comparable. No worksheet. (Gee, 7/26/04)

See also 52093 - 079 Record 156317 for a similar study on inter-observer reliability, 11/4/94, Bio-Research project 29539, submitted for cyclanilide and reviewed by Aldous, 10/3/97.

OTHER INFORMATION

169-390 170647 " Range-finding toxicity study in rats with carbaryl technical." (N. N. Hamada, Hazleton Laboratories America, Inc., Vienna, VA, HLA 656-137, 9/10/90). Carbaryl technical, lot 87191, 99.3% purity, was fed in the diet to Crl:CD7BR rats, 10/sex/group, as follows. Main study animals received 0, 50, 125, 500/4500, 1500/6000 or 3000 ppm for six weeks. The doses were increased after 3 weeks due to a lack of a compound effect. In the supplemental study, 10/sex/group received 0, 300, 6000, 12000 or 24000 ppm in the diet for 4 weeks followed by an extension for an additional 4 weeks (total of 8 weeks) due to a lack of sufficient treatment-related effects. In the main study, hematology and clinical chemistry parameters were measured including plasma, erythrocyte and brain cholinesterase at week 6. In the supplemental study, only cholinesterases were assayed at termination. Body weights were lower at $\geq$ 3000 ppm. Clinical signs were noted at 4500 and above including thin appearance, urine stains, hunched appearance and rough haircoat. At 12000 ppm, 3 females were found dead or sacrificed and at 24000 ppm, 1/sex were found dead. No cause of death was reported by the author but from clinical observations, mortality was probably treatment-related. The NOEL for cholinesterase inhibition was 300 ppm. Systemic NOEL = 1500 ppm (decreased body weight). Supplemental range-finding study. (Gee, 9/30/99)

023, 038359, 038177 and 038178; 1961 review article in Agricultural and Food Chemistry, 9:30-39, by Carpenter et al, summarizing: chronic rat and dog studies, Mellon reports #21-88 and #21-89, in -099, 719 and 718, respectively; mouse subcutaneous oncogenicity, Mellon report not on file; hen neurotoxicity study, Mellon report #21-87, in -134, 37928.

167, 050443; Exact duplicate of 169 - 023 above.

169 - 167, 050446 & -47: Journal article - Weil et al (1972). Current status of tests of carbaryl for reproductive and teratogenic effects. Toxicol. Appl. Pharmacal. 21:390-404. Covers rat reproduction and teratology studies in Mellon Institute reports:
#28-53, teratology and reproduction, in -099, 716;
#29-49, teratology, in -099, 715;
#35-65, reproduction (through F_{1a} only), in -099, 712. (No worksheet).

167, 050448 & -49: Journal article - Weil et al (1973). Comparative effect of carbaryl on rat reproduction and guinea pig teratology when fed either [sic] in the diet or by stomach intubation. in Toxicol. Appl. Pharmacal. 26:621-638. Covers rat reproduction/teratology/dominant lethal and guinea pig teratology studies in Mellon Institute reports:
#35-65, rat reproduction (all generations), teratology, and dominant lethal, in -099, 712,

27204, and 27202, respectively;
#34-81, guinea pig teratology, in -099, 713.
(No Worksheet).

023, 038365: One sentence summary of Mellon Institute chronic dog report #21-89, in -099, 718. Source of summary unknown.

023, 038363: One sentence summary of Mellon Institute rat reproduction report #28-53, in -099, #716. Source of summary unknown,

023, 038364: Two paragraph summary of Mellon Institute rat teratology report #29-49 and guinea pig teratology report #34-81, in -099, 716 or 713, respectively. Source of summary unknown.

023, 038366 and 038367: One paragraph summary of Mellon Institute rat reproduction report #35-65, in -099, 000712. Source of summary unknown.

169 - 155, 050427 Exact duplicate of 169-148, 046798, reviewed 10/27/86 by F. Martz. This is a literature review that states carbaryl is a primary neurotoxicant and cites 40 reports on acute toxicity in various species. Data from the open literature indicate that carbaryl can produce developmental toxicity and in some instances, teratogenicity. D. Shimer, 3/24/87.
148, 046798, Exact duplicate of 169-155, 050427, reviewed 3/24/87 by D. Shimer; Literature review of carbaryl entitled "Carbaryl: A Toxicological Review and Risk Analysis," by Morris F. Cranmer, in NeuroToxicology 7:247-332, 1986. This review article concerns material from published articles as well as unpublished information supplied by Union Carbide. Areas covered include neurotoxicity, acute toxicity, developmental toxicity, mutagenicity, immunotoxicity, oncogenicity, human exposure and toxicity, and cancer risk assessment of N-nitrosocarbaryl exposure. Twelve pages of references are provided which include the unpublished Union Carbide reports. F. Martz, 10/27/86.

55, Tab B, no record #; Correspondence dated 5/13/85 from EPA (Douglas D. Campt) to Union Carbide (J.S. Lovell) concerning carbaryl registration standard (no worksheet).

169 - 155, 050428 A review article on carbaryl entitled "Carbaryl: A Literature Review," by Michael E. Mont and Frederick W. Oehme, in Residue Reviews 80:1-6-4, 1981. Topics covered include chemistry, toxicity in target and non-target pests, birds, mammals, and humans, metabolism/ pharmacokinetics, environmental fate, interactions, analytical methods, and residues. Not reviewed in detail, no worksheet. F. Martz, 5/12/87.

55, 050429 Incomplete copy of EPA's Registration Standard for carbaryl, a partial duplicate of that document located in 169-103, 12895 (no worksheet). F. Martz, 5/12/87.

103, 012895; Contains the Regulatory Position section of the EPA Carbaryl Registration Standard submitted to CDFA by Union Carbide, and is a partial duplicate of material located in 169-155, 50429 (no worksheet). J. Schreider, 5/6/85.

SUBCHRONIC (4-week), DERMAL, RAT

169 - 413 186206 Austin, E. W. "4 Week repeated-dose dermal toxicity study with carbaryl technical in rats." (Covance Laboratories, Covance 6224-268, 3/8/02) Carbaryl

technical (99.49%) was applied to the skin of Crl:CD®(SD)IGS BR rats, 10/sex/group, at 0 (reverse osmosis water), 20, 50 or 100 mg/kg/day, 6-7 hours per day, 5 days per week, for 4 weeks. The test material was applied to moistened skin, under gauze, to approximately 10% of the body surface. The purpose of the study was to evaluate red blood cell and brain cholinesterase activity following dermal application. Body weight, food consumption and dermal effects were evaluated. A slight atonia was noted on occasion in 1/10 males and 4/10 females ($p = 0.043$) at 100 mg/kg/day. Body weight gain was statistically significantly lower days 5-12 in males at 100 mg/kg, being 24 $\pm$ 7.7 versus 33 $\pm$6.9 in control males. Total body weights, day 12, in males were not significantly different. RBC cholinesterase was evaluated before the daily application on days 1, 8, 15, and 22 and within 1 hour after dosing removal on days 5, 12, 19 and 26. Brain cholinesterase was determined in the right half of the brain following sacrifice on day 26. The method used for cholinesterase determination was not cited. The mean RBC activity was 10 to 15% lower than controls, especially in samples following dosing. At day 26, there were no differences among the control and treatment groups for RBCs. The mean brain cholinesterase activity, day 26, was 15% lower at 50 and 100 mg/kg/day in males and 24% lower at 100 mg/kg/day in females. Cholinesterase NOEL = 20 mg/kg/day. Dermal NOEL = 100 mg/kg/day in males and 50 mg/kg/day in females (atonia). Supplemental data. (Gee, 4/11/02) (Revised
7/23/04, Gee, for male body weight effect)

169 - 414 186207 Austin, E. W. "4 Week repeated-dose dermal toxicity study with SEVIN® XLR PLUS in rats." (Covance Laboratories, Covance 6224-267, 3/7/02) Sevin XLR Plus, 44.82% (wt/wt) was applied to approximately 10% of the body surface of Crl:CD®(SD)IGS BR rats, 8/sex/group, at 0 (water), 20, 50 or 100 µl/kg/day, 6-7 hours/day, 5 days/week, for 4 weeks. The test material was applied neat to the skin and covered. The purpose of the study was to evaluate red blood cell cholinesterase activity following dermal exposure. 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 hour after dosing removal on days 5, 12, 19 and 26. No brain cholinesterase activity was measured. No necropsy was performed. In females at 100 µl/kg/day, there was a 12% inhibition (statistically significant at $p \leq 0.05$) on days 5 and 12 after dosing but not on days 19 and 26, which were comparable with controls. In males at the high dose, there were no samples with significant inhibition. Due to the mild inhibition and the lack of consistency, the relationship of the inhibition to test article administration was uncertain. The method used for cholinesterase activity was not cited and the selection of doses not justified. The NOAEL = 100 µl/kg/day with a clear NOEL not established. Supplemental data. (Gee, 4/11/02).

169 - 415 186208 Austin, E. W. "4 Week repeated-dose dermal toxicity study with SEVIN® 80S in rats." (Covance Laboratories, Covance 6224-266, 3/8/02) Sevin 80S (lot C8I168025A, 80.07%) was applied to approximately 10% of the body surface of Crl:CD®(SD)IGS BR rats, 8/sex/group, at doses of 0 (reverse osmosis water), 20, 50 or 100 mg/kg/day, 6-7 hours per day, 5 days/week, for 4 weeks. The material was applied as a powder to moistened skin and covered. The purpose was to evaluate red blood cell cholinesterase activity. Body weight, food consumption, dermal irritation and clinical signs were evaluated and there were no treatment-related findings. RBC cholinesterase activity was measured pretest, before dosing on days 1, 8, 15 and 22 and within 1 hour after removal of the dosing material on days 5, 12, 19 and 26. No brain cholinesterase was measured and no necropsy performed. RBC cholinesterase activity was inhibited (8 to 20%) at 50 and 100 mg/kg when samples were taken within the hour after dosing. With samples taken before dose, there was no consistent pattern of inhibition. Cholinesterase NOEL = 20 mg/kg/day. The method of cholinesterase

analysis was not cited and the doses were not justified. Supplemental data. (Gee, 4/11/02)

SUBCHRONIC (4-week), ORAL, MOUSE

169-401 177758 "Carbaryl - Preliminary 28-day toxicity study in the male TSG p53 wild type mouse by dietary administration." (M. Dange, Rhone-Poulenc Agro, Studies SA 97499 and SA 97538, April 10, 1998). Carbaryl (batch 208115110, 98.4%) was fed in the diet to C57BL/6 TSG p53 wild type male mice, 6 weeks of age at start of dosing. In study SA 97499, diets of 0, 160, 1000 or 8000 ppm were fed to 10 mice per group. In study SA 97538, diets of 0, 2000 or 4000 ppm were fed to 10 male mice per group. Mean achieved intake in mg/kg/day was, with increasing diet concentration, 0, 35.7, 222, 424.4, 935.6 and 2107.3. On day 29 or 30, all mice were necropsied. The organ weights for liver and kidneys were recorded. No histopathology was performed. The primary data collected were for body weight, mortality, clinical signs and organ weight. No clinical signs or mortality were noted in either study. The major results were for body weight. At 8000 ppm, mice lost about 14% of their initial body weight in the first week (20.16** versus 23.26) and did not recover by the end of the study. At 4000 ppm, mice had lower body weights between 5.5 and 8.5% (mean of 6.5%) over the 4 weeks with a slight loss in week 1 (21.19** versus 21.54). No effect on body weight was seen at 2000 ppm or lower. The other significant effect was on the relative liver weight at 8000 ppm, which was statistically higher than controls (+ 15%) but absolute organ weights were comparable. The relative liver weights for 4000 ppm (+ 11%**) and 2000 ppm (+ 5%*) were also higher, but not the absolute weights. No treatment-related macroscopic organ findings were seen at necropsy. Supplemental study. No worksheet. (Gee, 2/26/04)

ACUTE ORAL, RAT

169 - 0463 212610 "Research report: A study on the effect of substrate concentration and detection wavelength on the blood cholinesterase assay in the rat." (Brooks, W., study director, Bio-Research Laboratories, Ltd., 10/19/95, project 29803) Male rats (5/group) were given vehicle or 30 mg/kg carbaryl (technical grade, 99.1%) in a single dose by oral gavage. Blood was sampled at 45-50 minutes (estimated peak of activity) and whole blood and plasma cholinesterase activities were determined. The assay was conducted at either 480 or 405 nm and the substrate was used as supplied or diluted 1:5 with water. Results indicated that the absolute enzymatic activity (U/L) increased 4.3 to 6 fold when the wavelength was changed from 480 nm to 405 nm (both undiluted and diluted substrate) but the ratio of test samples to controls did not change significantly with change in wavelength. Dilution of the substrate had no significant effect at either wavelength. Hematocrit was used to calculate the RBC cholinesterase activity. Supplemental study. (Gee, 7/20/04)

169 - 339 142599 "An acute benchmark-dose toxicity study of orally administered carbaryl, technical grade, in rats." (Brooks, W. and B. Broxup, Bio-Research Laboratories, Quebec, Project 97387, October 12, 1995) The benchmark dose was defined as the highest non-lethal dose. Carbaryl (lot 201085006, 99.1%) was given by gavage to 2/sex Sprague-Dawley Crl:CD®(SD)BR rats at 10, 50, 100, 250, 500 or 1000 mg/kg in 0.5% (w/v) carboxy-methylcellulose/0.1% Tween 80, in a single dose at 10 ml/kg. Dosing was followed by a three-day observational period for clinical signs/mortality. Body weights were recorded on days 0, 1 and 3. No necropsy was performed. Analytical data for test article were included. A detailed physical exam was performed on day 0 predosing and at 0.5, 1, 2, 4 and 8 hours post dosing

and on days 1, 2 and 3. Results: At 1000 mg/kg, all animals were found dead within 24 hours. At 500 mg/kg, 1/2 males and 2/2 females were found dead within 24 hours. All animals survived at 250 mg/kg = benchmark dose. Clinical observations were seen in all groups above 10 mg/kg/day. Within 30 minutes, both sexes, all rats at ≥ 50 mg/kg exhibited slight to severe salivation, tremors of head, body and/or limbs. Additional observations seen in some or all groups at 50 mg/kg and above included lacrimation, periorbital staining, urogenital staining, decreased activity, decreased respiration rate, abnormal breathing sounds and weakness. With a few exceptions, such as staining, decreased activity and weakness, many of the signs were no longer observed 1 day after dosing. All groups except 10 mg/kg showed weight loss between day 0 and day 1. Supplemental study. No worksheet. (Gee, 2/26/04)

169-338, 464 142593, 212611 " A time of peak effects study of a single orally administered dose of carbaryl, technical grade, in rats." (Brooks, W. and B. Broxup, Bio-Research Laboratories, Quebec, Project 97388, October 12, 1995) Carbaryl (lot 201085006, 99.1%) was given in a single oral dose by gavage at 0 (0.5% carboxymethylcellulose/0.1% Tween 80), 10, 50 or 125 mg/kg to Sprague-Dawley (Crl:CD®(SD)BR) rats of both sexes. There were three/sex/dose in the behavioral phase with termination after 24 hours and 15/sex/dose in the cholinesterase phase with 3/sex terminated at 0.5, 1, 2, 4, or 8 hours post dosing. In the behavioral phase, animals were given an abbreviated FOB predose and at 0.5, 1, 2, 4, 6, 8 and 24 hours after dosing. The FOB consisted of observations in an arena, which included locomotor activity, gait, tremor, twitches, convulsions, behavior, respiratory rate, lacrimation, salivation, staining and diarrhea. These animals were evaluated for cholinesterase activity at 24 hours. The cholinesterase activity of whole blood, plasma and whole brain was measured. The RBC activity was calculated. FOB findings were noted at 50 and 125 mg/kg whereas only 1 male at 10 mg/kg showed muzzle staining prior to sacrifice at 0.5 hours. Some FOB findings decreased in frequency and/or severity with time after dosing. By 8 hours, at 50 mg/kg in males, one animal showed muzzle and urinary staining only. At 125 mg/kg, 8 hours, males showed lacrimation, muzzle and urinary staining. By 8 hours in females, muzzle staining and urinary staining were still present at 50 and 125 mg/kg. At 24 hours, muzzle and urinary staining were still visible in both sexes at 125 mg/kg. The NOEL for clinical signs/FOB = 10 mg/kg, both sexes. Evaluation of the cholinesterase activity indicated that the time of peak difference in activity was at 0.5 and 1 hour. By 2 hours, whole blood activity at 10 mg/kg was comparable to controls. At 50 mg/kg, activity in females was comparable to controls by 4 hours, whether compared to concurrent vehicle controls or to predose values. Recovery was slightly slower in males. Activities in whole blood and plasma were comparable to controls at 24 hours. At 125 mg/kg, all activities were still lower than controls at 24 hours. In the brain, activity was comparable to controls at 24 hours for 10 and 50 mg/kg but still lower at 125 mg/kg, being 77% of control in males and 65% in females. Since there were only 3/sex at each sampling time, statistics were not done. Data for the hematocrits used to calculate the RBC activity were submitted in 169-464. The conclusion was that the times of peak effects were at 0.5 and 1 hour, based especially on the results at the lower doses. Supplemental study. (Gee, 2/27/04)

169 - 340, 467 142600, 212615 " An acute study of the time course of cholinesterase inhibition by orally administered carbaryl, technical grade, in the rat." (Brooks, W. and B. Broxup, Bio-Research Laboratories, Quebec, Project 97392, October 23, 1995) Carbaryl, technical grade, lot 201085006, 99.1%, was given by oral gavage in a single dose at 0 (0.5% carboxymethylcellulose/0.1% Tween 80), 10, 30 or 90 mg/kg in 10 ml/kg, to male and female Sprague-Dawley (Crl:CDMBOL 226 \f "Symbol" \s 11(SD)BR) rats. There were 24 per dose group with 6/sex/dose sacrificed at 1, 8, 24 or 48 hours 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. The regions of brain were: left hemisphere for whole brain, and regions from the right hemisphere were frontal cortex, hippocampus, cerebellum and caudate/putamen. Clinical signs were recorded. No clinical signs were reported for 10 mg/kg animals. 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 observation at 90 mg/kg up to 2 days (termination of the study). NOEL = 10 mg/kg, based on clinical signs. At one-hour post dose, cholinesterase activity was statistically lower in all samples from the 30 and 90 mg/kg groups and in most samples at 10 mg/kg. By 8 hours, all samples at 10 mg/kg were comparable to control and by 24 hours, all samples from 30 were comparable to controls. By 48 hours, all samples were also comparable to controls. ChE NOEL < 10 mg/kg at 1 hour, 10 mg/kg at 8 hours, 30 mg/kg at 24 hours and $\geq$ 90 mg/kg at 48 hours. The data for the individual hematocrits used in calculating the RBC activity submitted in 467. Supplemental study. (Gee, 2/27/04 and 7/26/04)

METABOLISM

** 169 - 0453 209656 " Metabolism of ^{14}C-carbaryl in rats (preliminary and definitive phases)." (Struble, Craig B., Hazleton Wisconsin, HWI 6224-184, R013850, August 5, 1994) ^{14}C-Carbaryl was given to HSD:SD rats at a low dose of approximately 1 mg/kg (Groups A, B and C) and a high dose of 50 mg/kg (Group D) with 5 per sex per group. The low dose was given to Group A by iv, to Groups B and C by oral gavage and the high dose by oral gavage. Group C had received 14 days of dosing with non-labeled carbaryl prior to the radio-labeled carbaryl. Results of analyses of the urine and feces indicated that metabolism was similar for both sexes and doses. Mass balance for all dose groups ranged from 97.6% to 104% for males and 96.1 to 103% for females. Comparison with the iv group indicated approximately 95% or greater absorption. Urine was the primary route of excretion with 84.5% to 95.0% [including cage wash/wipe] of the administered dose and with 6.98% to 12.5% in the feces. None was found in CO_2. Less than or equal to 0.91% of the administered dose was found in the carcass plus tissues. Most of the carbaryl was excreted within 12 hours for the low dose and 24 hours for the high dose. Metabolites were identified with reference standards using TLC, HPLC and LC/MS. Four conjugated metabolites were found in urine. Identified metabolites accounted for 75% in urine and 1% in feces, with the major one in feces being dihydro-dihydroxy carbaryl. Three major pathways were observed: arene oxide formation with metabolism to dihydro-dihydroxy carbaryl and conjugation, carbamate hydrolysis to form 1-naphthol and oxidation of N-methyl moiety. A metabolic pathway was proposed. ACCEPTABLE. (Gee, 3/3/04)

160 - 0454 209657 " Carbaryl: Investigation of the metabolism of [^{14}C]-carbaryl in the 15 month-old male rat following chronic dietary administration, final report." (Totis, M, Rhone-Poulenc Agrochimie, study SA 95288, R014082, December 19, 1996, amended on October 3, 1997) CD male rats, 15 months of age at initiation of dosing, were divided into 5 groups: Group A, single dose of 50 mg/kg [^{14}C]-carbaryl and followed for 168 hours with urine and feces collected; Group B, fed control diet for 83 days followed by 7 daily doses of labelled carbaryl at approximately 2 mg/kg; Groups C and D, fed at 250 ppm or 7500 ppm in the diet followed by 7 daily doses of labelled carbaryl at approximately 2 mg/kg and Group E, added later, and fed at 1500 ppm for 83 days followed by the 7 doses of radioactive material. Achieved doses were 9.89, 250.71 and 58.96 mg/kg/day over the 13 weeks of feeding. Five males in groups B, C, D and E were fed these doses for 90 days and were used for histopathological examination and for biochemical analyses for total glutathione, protein, glutathione peroxidase and glutathione-S-transferase in the liver. There were 23 metabolites in urine and twenty in feces, including carbaryl. In urine, the major components were UMET/11 (Glucuronide of dihydro-dihydroxy carbaryl), UMET/18 (α-Naphthyl β-D-glucuronide, sodium salt) and UMET/23 (sulfo conjugate

of naphthol). The major portion of the administered dose was excreted in the urine within the first 24 - 48 hours with greater than 65% of administered doses. The feces contained considerably less radioactivity with some carbaryl found as well as a number of metabolites (not identified). Tissue levels were low with the kidneys, in general, containing the most residual activity. Terminal body weights at 7500 ppm were significantly lower than controls with an increase in liver weight. Histopathology of the livers indicated centrilobular hypertrophy, pericholangitis and a tendency toward bile duct hyperplasia at 7500 ppm. Follicular cell hypertrophy was seen in the thyroid in 3, 5 and 5 rats (N = 5) in 250 through 7500 ppm. The conclusion was that 15 - 18 month old male rats are capable of significant metabolism of carbaryl, and are similar to young rats [see record 209656]. Supplemental study. (Gee, 3/8/04)

169 - 0455 209658 "Carbaryl: Liver cytochrome P-450 inducer phenotyping in the male CD1 mouse." (Thomas, H., Ciba-Geigy Limited, Basel, Report CB 94/23, R013827, October 21, 1994) Livers were from mice treated with 0 or 8000 ppm (1154 mg/kg) in the diet for 14 days (Groups 5 and 4 of record 209659 above under "DNA damage/repair"). Frozen livers were thawed, homogenized and cytosolic and microsomal fractions obtained by centrifugation. The protein content of both fractions was determined. The following parameters were compared: Cytochrome P-450, 7-ethoxyresorufin o-de-ethylase (EROD), 7-pentoxyresorufin o-depentylase (PROD), regio- and stereoselective testosterone hydroxylation and glutathione content. Results: Body weights in treated mice were 85% (28.88) of controls (34.08) and relative liver weights were increased to 135% of controls. Microsomal protein was increased to 132% of controls. Cytochrome P-450 was elevated to 1.3 of control (15.13 nmol/min/g liver* versus 11.21), EROD increased to 1.9 of control, PROD to 3.1, and total testosterone hydroxylation was elevated 152% (86.69* versus 56.95 nmol/min/g liver). The slightly increased level of glutathione did not reach statistical significance. In comparison, carbaryl represented a weak barbiturate-type inducer of cytochrome P-450 system in male mice. Supplemental study. No worksheet. (Gee, 3/4/04)

169 - 402 177759 B. Valles "Carbaryl: Investigation of the metabolism of [^{14}C]-carbaryl following 14 days administration to the male CD_1 mouse." (Rhone-Poulenc, Sophia Antipolis, Study SA 97481, 6/16/99) Male CD_1 mice (10 per group) were fed diets containing 0, 10, 100, 1000 or 8000 ppm carbaryl for 14 days followed by a single dose of 50 mg/kg [^{14}C]-carbaryl by gavage on the fifteenth day. Urine and feces were collected at 24-hour intervals following dosing for 168 hours, after which the animals were sacrificed. Radioactivity in the carcass and blood was determined. The metabolites in pooled urine were quantified for 0-24, 24-48 and 48-96 hours. There were 21 components found in the urine with the four major components being the dihydro, dihydroxy-naphthyl sulfate, the hydroxy-carbaryl glucuronide, α-naphthyl sulfate and α-naphthyl β-D glucuronide. The first two, apparently formed by epoxide intermediates, were increased in the mice given 8000 ppm in the diet, suggesting that at high doses of carbaryl, the metabolism, distribution and excretion pattern may be altered with a higher proportion of reactive intermediates being formed. Comparison with results from the rat suggests there are some differences in metabolism, although a more complete study would be required to elucidate the metabolic pathway in mice. Supplemental study. (Gee, 7/8/04)

Appendix VII. Stakeholder comments

- **OEHHA comments and DPR response**
- **Bayer comments and DPR response**

(STAKEHOLDER COMMENTS & DPR RESPONSES START ON THE FOLLOWING PAGE)

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M E M O R A N D U M

TO: Gary T. Patterson, Ph.D., Chief
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FROM: Anna M. Fan, Ph.D., Chief
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DATE: July 22, 2013

SUBJECT: COMMENTS ON THE DRAFT RISK CHARACTERIZATION DOCUMENT FOR CARBARYL

The Office of Environmental Health Hazard Assessment (OEHHA) has reviewed the draft Risk Characterization Document (RCD) for occupational and ambient air exposure to carbaryl, prepared by the Department of Pesticide Regulation (DPR), dated July 12, 2012. Our comments are provided in the attachment. OEHHA is providing comments on the Exposure Assessment Document for Carbaryl separately. OEHHA reviews risk assessments prepared by DPR under the authority of Food and Agriculture Code section 11454.1.

OEHHA has provided a number of comments on the risk characterization methodology and conclusions of the draft RCD. These comments and our recommendations, as well as suggested clarifications, additions and corrections, are contained in the attachment.

Thank you for providing this draft document for our review. If you have any questions regarding OEHHA's comments, please contact Dr. David Ting at (510) 622-3226 or me at (510) 622-3200.

Attachment

cc: David Ting, Ph.D., D.A.B.T.
Chief, Pesticide and Food Toxicology Section
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Office of Environmental Health Hazard Assessment

OEHHA's Comments on DPR's Draft
Risk Characterization Document for Carbaryl
(Occupational and Bystander Exposures)

The Office of Environmental Health Hazard Assessment (OEHHA) is responding to a request from the Department of Pesticide Regulation (DPR) to comment on the draft Risk Characterization Document (RCD) for carbaryl dated July 12, 2012.

OEHHA reviews risk assessments prepared by DPR under the authority of the Food and Agricultural Code Section 11454.1, which requires OEHHA to conduct scientific peer reviews of DPR risk assessments.

SUMMARY

The RCD was well-written and comprehensive in the presentation of the toxicological studies, analysis of weight of evidence, and approaches used to derive the critical endpoints and points of departure (PODs) for the calculation of margins of exposure (MOEs) and cancer risk. It included a thorough discussion on the uncertainty associated with endpoint and PODs selection and the associated impact on the risk values (MOEs and oncogenic risk). Overall, OEHHA concurs with the selection of the studies and endpoints as well as the extrapolation methods. The main concern is with the dose-response analysis of the critical studies.

- For assessing inhalation toxicity hazard, results from oral toxicity studies were used because of inadequacy in the inhalation toxicity database. OEHHA finds this approach scientifically appropriate.

- For assessing dermal toxicity, the same POD was used for all durations. This POD was derived from a subchronic dermal toxicity study. OEHHA concurs with this approach.

- For assessing acute oral and inhalation toxicity, the POD of 1 milligram per kilogram-day (mg/kg-day), based on observation of the pregnant rats in a developmental neurotoxicity study of Robinson and Broxup (1997), was selected. OEHHA has concerns with this choice because there is sufficient evidence to support a POD of less than 1 mg/kg-day for the calculation of acute oral and inhalation MOEs. OEHHA recommends additional data analysis of the clinical signs in this study, and of mortality in pup dogs in a developmental toxicity study reported by Immings et al. (1969).

- Regarding carcinogenicity:
  - The RCD identified carbaryl as a carcinogen because carbaryl induces a number of different tumors in rat and mouse cancer bioassays. OEHHA agrees with the carcinogenicity identification.
  - The combined incidence data for hemangiomas and hemangiosarcomas in male mice were selected for cancer dose-response analysis using the multistage cancer model. OEHHA finds these approaches appropriate.
  - For determining acceptable risk, the RCD used a benchmark probability of cancer of one in a million, as is used by OEHHA.

- Regarding early age susceptibility:
  - For cancer, the RCD did not account for potential heightened sensitivity early in life from exposure to carcinogens. OEHHA recommends the incorporation of age-sensitivity factors (ASFs) to account for increased risk of cancer due to exposure to carbaryl during childhood.
  - For developmental toxicity, a POD was not determined for pup mortality observed in the study with dogs (Immings et al., 1969). A lowest-observed-effect level (LOEL) of 2 mg/kg-day was established. OEHHA is concerned about this endpoint and that a POD, when determined, may be lower than the POD selected for MOE calculations. If the data do not support a POD determination, an additional uncertainty factor should be considered to determine acceptable exposure.

- Regarding non-cancer dose response analysis:
  - The PODs, when derived from benchmark dose (BMD) analysis and used in the MOE calculations, were based on a benchmark response (BMR) of ten percent. OEHHA recommends the use of five percent as the BMR, especially for endpoints related to effects in the brain.
  - The risk of non-cancer effect from exposure was evaluated using the MOE approach and the benchmark was a value of 100 to determine acceptability of exposure. OEHHA agrees with these approaches.

The exposure assessment section of this draft RCD generally reflects the information from the draft Exposure Assessment Document (EAD). The OEHHA review of the draft EAD is provided in a separate memo.

GENERAL COMMENTS

The RCD addressed the following scenarios:

- Workers - inhalation and dermal exposures from agricultural and residential uses;
- Bystanders - inhalation exposure from agricultural and public pest control uses;

- Residents - dermal, inhalation, and oral (toddlers only) exposure from lawn care uses;
- Swimmers - oral and dermal exposures in contaminated water;
- Ambient air- inhalation

The RCD for dietary exposure to carbaryl (DPR, 2010[1]) was previously reviewed by OEHHA (January 29, 2009; OEHHA 2009a). In that review, OEHHA agreed with DPR's choice of critical studies and toxicological endpoints as the basis for all carbaryl risk calculations with one exception, the acute POD of 1 mg/kg-day based on decreased body weight gain and increased cholinergic signs. OEHHA recommended that DPR perform BMD extrapolation with several time points for change in gait and other endpoints such as pinpoint pupils or cholinesterase measures, and then decide on the appropriate POD for the evaluation of acute exposures. For the current draft RCD for occupational and residential exposures, OEHHA remains concerned about the selection of 1 mg/kg-day as the POD. This concern is explained further in this review.

For the endpoints analyzed by BMD in the draft RCD, all BMR were set primarily at 10 percent for both quantal and continuous data. The use of a 10 percent BMR for brain cholinesterase (ChE) inhibition was justified by the lack of overt clinical signs and histopathology, as discussed in a chronic toxicity study review (page 112). While DPR suggested that a lower BMR of 5 percent may be more appropriate for cholinergic signs (page 146), the modeling result using this BMR was not used in the calculation of the MOEs. OEHHA supports the consideration of 5 percent as the BMR for this endpoint. OEHHA typically uses a 5 percent BMR for the dose-response analysis of quantal data (OEHHA, 2008).

SPECIFIC COMMENTS

The following sections present OEHHA's comments relating to specific exposure routes and types of toxicity.

Inhalation Toxicity (Acute, Subchronic, and Chronic)

The draft RCD selected the critical no-observed-effect levels (NOELs) from oral studies to address inhalation exposure to carbaryl due to insufficient inhalation toxicology information. There were no subchronic or chronic inhalation toxicity studies. There was only one inhalation toxicity study with rats exposed to carbaryl for three hours at air concentrations ranging from 10 to 65 milligrams per cubic meters (mg/m^3) (page 40) (Weinberg, 2008). The lower-bound effective dose for BMR of 10% (LED_{10}) for brain cholinesterase inhibition was 9.81 mg/m^3 (equivalent of 1.18 mg/kg using a DPR default

[1] The draft RCD for dietary exposure was reviewed by OEHHA in 2009. The draft was finalized in 2010.

rat inhalation rate of 0.96 cubic meters per kilogram-day (m^3/kg-day). This study was considered inadequate because the rats were exposed for only 3 hours instead of 4 hours as recommended under U.S. Environmental Protection Agency (USEPA) guidelines. DPR used acute oral toxicity data to evaluate acute inhalation exposure and chronic oral data to evaluate subchronic and chronic inhalation exposures.

OEHHA concurs with the reasons stated for the use of oral toxicity studies for assessing inhalation toxicity in this case.

Dermal Toxicity (Acute, Subchronic, and Chronic)

This draft RCD selected a 4-week dermal study in rats (Austin, 2002a) as the critical study. It identified a LOEL of 50 mg/kg-day based on the observed inhibition of brain and red-blood cell (RBC) cholinesterase activities and a NOEL of 20 mg/kg-day. Among the dermal studies on systemic toxicity, it was the only study that was considered adequate, and was used for the evaluation of acute, subchronic, and chronic dermal exposures.

OEHHA concurs with use of the critical subchronic study to address acute, subchronic and chronic dermal exposure to carbaryl. OEHHA suggests using BMD analysis to better characterize the POD for MOE calculation.

Acute Oral Toxicity

For the evaluation of acute oral and inhalation exposures, DPR identified a POD of 1 mg/kg-day based on weight gain deficits, cholinergic signs, and brain and RBC ChE inhibition observed in pregnant rats at a LOEL of 10 mg/kg-day and a NOEL of 1 mg/kg-day from a neurodevelopmental rat study by Robinson and Broxup (1997). The draft RCD also determined an alternative POD of 0.25 mg/kg-day for slight hypotonic gait incidence data gathered during gestation. This value was derived from BMD modeling of the same study that resulted in a LED_{10} of 0.25 mg/kg-day (page108).

OEHHA has concerns about (1) the selection of 1 mg/kg-day as the POD and (2) the approach used to determine the LED_{10} of 0.25 mg/kg-day.

<u>(1) Selection of 1 mg/kg-day as the POD</u>

DPR chose to use the NOEL (1mg/kg-day) over the LED_{10} (0.25 mg/kg) as the POD to calculate the MOEs because according to their evaluation there is a stronger experimental support for the former value (page 112). In addition, the level was supported by the three additional acute oral gavage studies from the same laboratory (Brooks and Broxup, 1995a, b; Brooks et al., 1995). Each of these studies established

cholinergic LOELs at 10 mg/kg-day, but did not establish NOELs (page 144). Further support came from:

a) "*The rat acute gavage study of Moser (2007) which established an LED_{10} of 1.1 mg/kg based on brain ChE inhibition (as well as a second LED_{10} of 0.78 mg/kg based on RBC ChE inhibition) in postnatal day 11 animals; and*
b) *The acute inhalation toxicity study of Weinberg (2008), which established an LED_{10} of 1.18 mg/kg based on brain ChE inhibition."*

OEHHA suggests that DPR reconsider the selection of 1 mg/kg-day as the POD because additional data analysis is needed. The calculation of MOEs should be based on the most sensitive endpoint, represented by the lowest POD, which is 0.25 mg/kg-day in this draft of the RCD. PODs need to be determined for the three cited oral gavage studies with only LOELs established, for comparison. Brain and RBC ChE inhibition data should be analyzed using additional, possibly lower, BMRs, instead of a BMR of 10 percent. In addition, the acute inhalation toxicity study of Weinberg (2008) was not properly conducted, as stated in the draft RCD, and therefore this study should not be used to support a POD of 1 mg/kg-day.

<u>(2) Approach used to determine the LED of 0.25 mg/kg-day</u>

In the draft RCD, the value of 0.25 mg/kg-day, as an alternative POD, was obtained by using normalized mean incidence rate for hypotonic gait (Table III-16a, page 99). The following reason was provided for combining the gestational data set from gestation day 6 (GD 6) to gestation day 20 (GD 20):

> "*Because of carbaryl's propensity for clearance from the rat system in less than 24 hours (Struble, 1994) and the relatively rapid decarbamylation reaction (t0.5 = 40 min (cf., Cranmer, 1985)), all of the FOB (Functional Observational Battery) tests conducted during the gestation period were considered to represent separate, but equivalent, acute scenarios. 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 (p. 110)*."

OEHHA notes that the draft RCD also stated that this approach added uncertainty "*because it implied that data from single test days represented fluctuations around a mean…it remained possible that it underestimated the sensitivity of the system (page 146)*." OEHHA finds no basis for this assertion of random fluctuation and justification for the use of the average incidence. OEHHA agrees with the latter point that the use of the mean incidence rates would underestimate the toxicity of carbaryl on certain days of exposure. Examination of the data in Table III-16a (pages 99 to 100) showed consistent increased incidences of hypotonic gait for three consecutive observation

days (GD 12, 15, and 18) for the 1 and 10 mg/kg-day groups. This finding of multiple occurrences increases the significance of the observed clinical sign.

The draft RCD stated the following uncertainty surrounding the 0.25 mg/kg LED_{10} determination (page 145-146):

1. "*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*."…" *In six FOB tests conducted during gestation and five conducted within 21days 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*."

2. "…*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."…"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."*

3. The FOB parameters being used for this evaluation are "*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. There were uncertainties inherent in the BMD approach such as: (1) Choice of "*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%; (2) The decision to delete the top dose, which was made in order to generate a curve of appropriate fit added uncertainty since it ignored actual data gathered in the experiment; (3) The choice of the probit function over other algorithms added uncertainty because each algorithm generated different LED_{10} and ED_{10} values; and (4) 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."*

OEHHA believes that for the evaluation of acute oral and inhalation exposures, a single-day exposure is relevant and would argue against taking an average of the data collected over six different gestation days (point #2). Effects observed on GD9 and GD12 but not on GD6 may be explained by a higher sensitivity after GD6.

On the concern over the validity of hypotonic gait observation (point #3), this observation was supported by data showing other clinical signs such as pinpoint pupil and slight tremor, which may also be due to effects on the central nervous system, or cholinesterase inhibitory effect on the neuromuscular system. OEHHA recommends additional analysis of data for multiple clinical signs, as presented in Table III-16a (page 100 under "Signs") of the draft RCD.

OEHHA does not agree that each BMD model generating different LED_{10} and effective dose at BMR of 10% (ED_{10}) values adds additional uncertainty (point #4). The best fit model, both statistically and by visual inspection of the graph, should provide the most appropriate POD. The inclusion of results from other models (e.g., other curves or BMR values) would have been helpful for this review.

Thus, OEHHA believes that there is sufficient evidence presented in the draft RCD to support a POD lower than 1 mg/kg to evaluate hazards from acute exposures to carbaryl. OEHHA recommends additional analysis of the data for hypotonic gait and other multiple signs (Robinson and Broxup, 1997) and evaluating the observation for each gestation day independently. For this study and other studies, alternate BMRs other than the default 10 percent (for example, 5 percent as suggested in Point #4) should be considered before selecting the appropriate POD to address acute oral toxicity and as a surrogate value for acute inhalation toxicity.

Subchronic Oral Toxicity

OEHHA notes that the subchronic oral exposure to carbaryl was evaluated by DPR using the critical chronic oral LED_{10} value of 0.5 mg/kg-day for brain ChE inhibition (Hamada, 1987) from the one-year chronic dietary dog study (pages 97, 112). The only subchronic dietary study available (Hamada 1991) had a NOEL (highest dose tested) of 3.83 mg/kg-day (5-week, page 44). According to the draft RCD, this value is considerably higher than the critical acute oral values (1 mg/kg or 0.25 mg/kg) and DPR considered it prudent to base the seasonal risk estimation on a value closer to the acute values.

OEHHA agrees with the use of the chronic oral toxicity study to address subchronic oral exposure, but suggests DPR consider OEHHA's recommendations to use 5 percent BMR for BMD analysis.

Chronic Oral Toxicity

The POD determined for chronic oral exposure was 0.5 mg/kg-day. It was derived from an LED_{10} of 0.5 mg/kg-day based on the brain ChE inhibition reported in a one-year chronic dietary dog study (Hamada, 1987). This value was used by DPR to evaluate the non-cancer risk from annual (i.e., chronic) oral exposure. OEHHA (2009a) has previously provided comments on the chronic oral risk assessment for the use of this LED_{10} value based on the one-year dog study (Hamada, 1987).

There were 3 other chronic/oncogenicity dietary studies with higher NOEL values:

(1) Rat 2-year chronic/oncogenicity study by Hamada (1993a) (NOEL: 10.0-12.6 mg/kg-day based on inhibition of brain AChE and reduced female weight gain),
(2) Mouse 2-year chronic/oncogenicity study by Hamada (1993b) (NOEL: (Male) 14.73 mg/kg-day; (Female) 18.11 mg/kg-day for presence of intracytoplasmic droplets/pigment in the bladders of both sexes, and inhibition of brain and RBC ChE), and
(3) Mouse 180-day oncogenicity study by Chuzel (1999) (NOEL: 5.2 mg/kg-day for globular deposits in the umbrella cell layer of urinary bladder).

OEHHA agrees with the use of the selected toxicity data to address chronic oral exposure, but suggests DPR consider OEHHA's recommendations to use BMR of 5 percent for BMD analysis.

Genotoxicity

In the draft RCD, the genotoxicity database was presented as a summary and in Table III-II (pages 70-71). The reader was referred to DPR's RCD for dietary exposure (DPR, 2010) for more detailed information. Carbaryl was considered a potential genotoxic compound because of positive results for genotoxicity in one of five gene mutation studies, four of six chromosomal aberrations studies and two of four DNA damages studies (page 114). Two metabolites of carbaryl, nitrocarbaryl and 1-naphthol, were reported to show some genotoxic activity (page 114). No new data were presented in this draft RCD.

OEHHA concurs with DPR's determination that carbaryl is potentially genotoxic. This section should be updated with new information, if any, relevant to the genotoxicity of carbaryl.

Oncogenicity

DPR concluded that carbaryl was carcinogenic because it induced a number of different tumors in rat and mouse cancer bioassays. This conclusion was based on the same

database as that presented in the dietary RCD. The potency was estimated using incidences of hemangiomas and hemangiosarcomas in male mice after dietary exposure to carbaryl for two years (Hamada, 1993b; Table III-8c, page 63). The database presented was the same as that in the dietary RCD (DPR, 2010). There was no new oncogenicity information.

In the review of the previous dietary RCD (DPR, 2010), OEHHA (2009a) had concurred with DPR's conclusion that carbaryl is a potential human carcinogen. OEHHA supported the use of the mouse tumor data (Hamada, 1993b) for potency calculation and the cancer potency factor based on the animal doses using the quantal linear model reported in the document. In this draft RCD, the cancer potency factor was recalculated using human equivalent doses in the multistage-cancer model. While OEHHA agrees with the approach, there appeared to be an error in the conversion factor used for converting mouse "internal" doses of 0, 14.73, 145.99 and 1248.93 mg/kg-day to human doses of 0, 2.12, 21.02 and 179.85 mg/kg-day (page 116). According to the equation on page 116, a conversion factor of 0.153 should have been used. However, a factor of 0.144 was actually used instead (e.g., 2.12/14.73 = 0.144). If the latter conversion factor value is correct, the reported human oncogenic potency of $1x10^{-2}$ mg/kg-day^{-1} and oncogenic risk estimates in the draft RCD will have to be revised. In addition, the use of the adjective "internal" to describe the doses may not be appropriate, unless the internal dose of carbaryl were actually measured or estimated by modeling. On page 58, these same doses were referred to as "systemic" doses, corresponding to the concentrations in the diet. There was no explanation how the part- per-million (ppm) values were converted to these dosage terms. It would be helpful to clarify this issue.

Reproductive and Developmental Toxicity

The draft RCD had an extensive discussion of the reproductive and developmental toxicity studies of carbaryl. In the Reproductive Toxicity section (pages 73 to 78), human epidemiologic studies conducted with carbaryl workers in factory and those exposed as a result of carbaryl use in farms were described. The results showed that carbaryl exposure was associated with increased spermatogenic toxicity (Wyrobek et al., 1981), sperm chromosomal aberrations and DNA damage (Xia et al. 2005), and miscarriages (Savitz et al., 1997). However, the exposure levels of the workers were not reported by the studies. This section also discussed the results of a 2-generation reproductive toxicity study with rats and repeated exposure studies conducted with rats and gerbil (pages 78 to 87). As summarized in Table III-13 (page 88), reproductive toxicity of carbaryl at the LOEL included: increased second generation (F_2) pup mortality (Tyl et al., 2001), decreased sperm motility (Shtenberg and Rybakova, 1968), changes in testicular enzyme levels, sperm and testicular histopathology (Pant et al., 1995), sperm abnormalities (decreased sperm count and motility, abnormal sperm)

(Pant et al., 1996), and decreased number of liveborn pups, pup survival, and weaning weight (Collins et al., 1971). The lowest NOEL was less than 7 mg/kg-day for effects in sperm (Shtenberg and Rybakova, 1968).

The Developmental Toxicity section described studies with results on developmental toxicity in several laboratory animals (rats, rabbits, mice, and beagle dogs). As summarized in Table III-15 (page 96), developmental toxicity of carbaryl included: reduced fetal body weights (Murray et al., 1979; Repetto-Larsay, 1998; Tyl et al., 1999), delayed ossification (Repetto-Larsay, 1998), teratogenic abnormalities (Smalley et al., 1968), and increased stillbirths (Immings et al., 1969). The lowest NOEL was less than 2 mg/kg-day for increased stillbirths in a study conducted with dogs (Immings et al., 1969).

Based on results from the reviewed studies, the draft RCD concluded that exposure to carbaryl can potentially lead to reproductive and developmental toxicity (page 156). OEHHA concurs with this conclusion.

In the context of comparison of PODs to address acute oral toxicity, the RCD discussed the results of the two developmental toxicity studies conducted with dogs (Immings et al., 1969; Smalley et al., 1968) (page 111). In the Smalley et al. study (1968), pregnant beagle dogs were exposed to carbaryl in the diet from GD 3 to parturition (GD 62). Increased incidences of pups with various teratogenic abnormalities (abdominal-thoracic fissures, branchygnathia, ecaudate pups, failure of skeletal formation, failure of liver development, and superfluous phalanges) were reported. The LOEL and NOEL were 6.25 mg/kg-day and 3.12 mg/kg-day, respectively.

No developmental toxicity NOEL was established for the second developmental toxicity study conducted in dogs (Immings et al., 1969). Pregnant beagle dogs were given carbaryl (0, 2, 5 or 12.5 mg/kg-day) in the diet from GD 1, continuing through weaning at 6 weeks of age. No treatment-related maternal effects were observed and the maternal NOEL was established at greater than 12.5 mg/kg-day. For the pups, the significant findings included increased stillborn and pup death (Table III-14). The LOEL was established at 2 mg/kg-day for non-statistically significant increase in stillbirths at that dose. The draft RCD noted that “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.” Furthermore, increased pup death (on per pup basis) at weaning was statistically significant for all doses (page 95). At 2 mg/kg-day, the percentages of pup deaths were 36 to 40 percent, compared to 12 percent for the control.

OEHHA supports DPR’s consideration of results from the dog studies for POD determination. However, OEHHA suggests that a POD also be determined for

increased pup mortality from the Immings et al. (1969) study. This POD would be compared to other PODs of the same exposure route and duration to insure that the selected POD for MOE calculations would be protective of the reproductive and developmental toxicity from carbaryl exposure.

Developmental Neurotoxicity

In the developmental neurotoxicity study conducted by Robinson and Broxup (1997), pregnant Sprague-Dawley rats were given carbaryl by gavage at 0, 0.1, 1, or 10 mg/kg-day from GD 6 to postpartum day 10 (pages 97-100). In the dams, decreased body weight gain, alterations in FOB measures, and inhibition of plasma, RBC and brain cholinesterase activity were seen at the 10 mg/kg-day dose level. In the offspring, alterations in brain morphometric measurements were observed in the 10 mg/kg-day dose group (page 98). DPR considered the changes in brain morphometry observed in the offspring inconsistent in degree and direction, and therefore established 10 mg/kg-day as the NOEL for developmental effects.

OEHHA disagrees with DPR's analysis of the brain morphometry data. Because of the morphological and physiological differences, different brain regions or different genders should not be expected to react to a toxicant in the same degree or direction. The bilateral decrease in the size of the forebrain (line A) in first generation (F_1) male adults and the bilateral decrease in cerebellar length (line F) in F_1 female pups are indicative of adverse effects of carbaryl on brain development. The appropriate LOEL is 10 mg/kg-day for the toxicity in the offspring, and the appropriate NOEL is 1 mg/kg-day. USEPA also identified 10 mg/kg-day as a LOAEL based on alterations in brain morphometric measurements in the Health Effects Division Chapter of the Reregistration Eligibility Decision Document for carbaryl (USEPA, 2007).

Pre- and Post-natal Sensitivity

DPR did not apply any additional uncertainty factor for potential increased sensitivity of infants and children to carbaryl in the previous dietary assessment (DPR, 2010) or this draft non-dietary risk assessment. In both documents, the rationale given was because the acute critical NOEL of 1 mg/kg-day was similar in magnitude to the POD of 1.1 mg/kg-day developed by USEPA (page 156). The USEPA POD was based on brain cholinesterase inhibition of young rats (postnatal day 11 rats) (Moser, 2007), and thus the sensitivity of young children had been taken into account (USEPA, 2007). However, this draft RCD appeared to suggest that an additional UF is needed. On page 158, it was stated that "*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 year subpopulations, it appeared that the extra 10-fold factor should be considered (page 158)*."

In the review of the dietary RCD (DPR, 2010), OEHHA supported DPR's decision not to apply an additional uncertainty factor to the MOE threshold of 100 for determining acceptable exposure, but recommended further investigation of the developmental effects observed in other studies, particularly in the dog (OEHHA, 2009a). In this draft RCD for non-dietary exposure, the results of the dog studies (Smalley et al., 1968 and Immings et al., 1969) were stated to indicate a potential developmental risk (page 156). While a NOEL was established for Smalley et al (1968), only a LOEL (2 mg/kg-day) was established for the Immings et al. (1969) study. OEHHA remains concerned that the PODs selected to calculate the MOEs may not be protective against the fetal mortality observed in the dog study (Immings et al., 1969). If a POD cannot be determined from the reported data in Immings et al (1969), then an additional uncertainty factor may need to be considered to account for the fact that the study only established a LOEL.

Aggregate Exposure Toxicity

The aggregate (multi-route) non-cancer health hazard was evaluated for occupational and residential exposures with the addition of dietary exposures. The only exception is that dietary exposure was not added to the inhalation exposure of the bystanders. The adult bystander exposure was used as the surrogate to represent the upper bound value of the general public's exposure to ambient air concentrations of carbaryl (page 143). For each exposure duration (acute and chronic), the total MOE was calculated using the reciprocal of the sum of the reciprocals of the dermal, inhalation, and oral MOE values:

$$MOE_{total} = 1 / [(1/MOE_{dermal})+(1/MOE_{inhalation})+(1/MOE_{oral})]$$

Since the endpoints for the PODs were all based on cholinesterase inhibition, OEHHA considers the use of the total MOE equation appropriate. However, rationale should be provided as to why dietary exposure was not considered in the aggregate exposure for adult bystanders, when this human receptor is used to represent the general public.

Dietary exposure was also not included in the estimation of oncogenic risk for all human receptors. The aggregate oncogenic risk was calculated only for the handlers, and it was the sum of risks from dermal and inhalation exposures (pages 132-134). The draft RCD stated that dietary risk was not added because the risk of 2.9×10^{-6} already exceeds the acceptable level of 10^{-6} (page 134, Table IV-7a, footnote d).

OEHHA agrees with using the total cancer risk approach to calculate aggregate lifetime exposures. However, the decision of whether to include the dietary component for the lifetime aggregate risk should be based on the probability of lifetime exposure to

carbaryl from multiple exposure pathways, rather than by the magnitude of the risk (> 10^{-6}) by the dietary route.

Margins of Exposure and Oncogenic Risk

For non-cancer hazard, the calculated MOEs for a single route and aggregate exposures were compared to a value of 100, which DPR considered to be protective of human health. This value reflected the default assumptions that (1) humans could be 10-fold more sensitive than animals and (2) that a 10-fold range of sensitivity exists within a human population (page 130).

OEHHA concurs with the use of a MOE of 100 as the threshold to evaluate non-cancer health risks, pending the additional consideration of the fetal mortality data in the dog study (discussed under Pre- and Post-natal Sensitivity in this document).

For cancer risk, DPR calculated lifetime exposures only for workers (dermal and inhalation routes) and for adult bystanders (inhalation route). DPR used a probability of one in a million for evaluating cancer risk. OEHHA concurs with the use of this level to evaluate cancer risk. However, OEHHA recommends that cancer risk should be calculated for the general population exposed to carbaryl in the ambient air. While ambient air concentration was not calculated in the EAD, the EAD indicated that the bystander exposure level could be used as an upper bound for an ambient air exposure level. OEHHA recommends that the lifetime exposure to carbaryl should include vulnerable periods such as third trimester in utero and early childhood. For this reason, OEHHA suggests the use of age-specific inhalation rates and ASFs (OEHHA, 2009b) in the calculation of lifetime inhalation exposure and cancer risk, respectively.

Editorial Comments

A list of tables would be helpful for the readers.

The information about the different exposure scenarios addressed in the draft RCD is not consistent with the actual scenarios evaluated in the document, and it needs to be revised. In various sections in the document, different scenarios were indicated, for example: 1) the title page indicates occupational and bystander exposures are addressed; 2) the Introduction section on page 119 listed occupational, bystander, and ambient scenarios; and 3) the second paragraph of the Risk Appraisal section (page 144) stated that the dietary exposure of both workers and the general public to carbaryl would be described.

Summary and Hazard Identification sections: It would be helpful to have a table summarizing the critical studies, PODs and endpoints used for the MOE and oncogenic

risk calculations in this section instead of at the end of the document (Table VII-1, page 164) as part of the reference dose and concentration presentation.

Page 41, Section III, Toxicology Profile: The summary table for acute toxicity is missing. A summary table for subchronic toxicity and chronic toxicity study was provided on page 69.

Page 42, Paragraph 1: According to paragraph 1, "Subchronic NOELs and LOELs are summarized in Table III-8." These values are actually summarized in Table III-10.

Page 43: The data in Moser (2007) have been published in Toxicological Sciences 114 (1):113-123 (2010). The results, if necessary, and the citation in the RCD should be updated.

Pages 45 and 69: According to the text, the systemic NOEL for the subchronic dermal Austin (2002a) study is 20 mg/kg-day. However, Table III-10 lists the systemic NOEL as 100 mg/kg-day. This inconsistency should be addressed.

Page 48, Paragraph 1: According to paragraph 1, "Chronic NOELs and LOELs are summarized in Table III-9." These values are actually summarized in Table III-10.

Page 87, Paragraph 6: Space should be provided between the title for Table III-13, "NOEL and LOEL values in laboratory animal studies on the reproductive toxicity of carbaryl" and the statement above so that the title appears on the top of the table.

Page 95: In the table, superscripts 3 and 4 should be c and d.

Page 98, Paragraph 3, the paragraph below "**F1 adults.** There were no clear effects of carbaryl in the F_1 adults.", which begins with, "The LOEL determination for maternal effects….At 10 mg/kg, very clear body weight gain decrements, RBC and brain cholinesterase inhibition…" appears to be referring to F_0 adults instead of F_1 adults because there was "no clear effects of carbaryl in the F1 adult" as mentioned above. Therefore, there should be a space between the description of F1 adult and the following paragraph, which should be titled: Conclusion.

Pages 99-100 in Table III-16a: There seems to be an error in the incidence for "Signs" for the gd20 1.0 mg/kg group. It is lower (10/26) than that (11/26) for hypotonic gait alone. The incidence for "Signs" should be the same or higher since it represents the number of animals with one or more signs.

Page 100, footnote d: The incidences appeared to be for animals with one or more signs, not only those with "more than one sign" as indicated in this footnote.

Page 111, Paragraph 3, line 2: Hamada reference should be “Hamada 1987” instead of “Hamada 1997”.

Page 116, the 2nd paragraph “…*Fourteen separate algorithms available in the USEPA version 1.3.2 benchmark dose…were compared as potential models for the male vascular tumor data…* ”: This paragraph was in the dietary RCD; it should be reviewed to see if it is still applicable for this RCD. As shown in Appendix III, the slope factor was calculated using the multistage cancer model from a more recent version (version 1.9) of the software. Multistage cancer model is the only appropriate model for the determination of potency. On this page and elsewhere, instead of “algorithm”, the conventional term is “model” in reference to the statistical models in the BMD software.

Page 117: Paragraph 2, line 2: Typographical error, “adquate” should be “adequate”.

Page 131, Paragraph 1, line 2: This line reads, “”hazard index” approach (footnote c, Table xxxg).” Please clarify which table is being referred to by Table xxxg. Also, location of Table xxxh on line 11 needs to be identified.

Pages 131-139: Mislabeling of the tables: xxxg, xxxh, xxxc.

Page 134, footnote c: The equation used to calculate the aggregate MOE is referred to as “Total MOE,” not “Hazard Index” as written in the footnote.

Page 134, footnote d: Aggregate oncogenic risk was the “sum”, not “product” as written in footnote.

Page 158, Paragraph 4, line 3: Which table is being referred to by “Table xxxl”.

Page 162, Footnote 18: the units are wrong: “m^3/mg/hr” should be “m^3/kg/hr”.

Page 179 (Appendix I): The description below the Probit Model stated, “Slight hypotonic gait data in males (top dose deleted)”. This note appears to be a typographical error since the title of this Appendix I, “Benchmark dose extrapolation for induction of slight hypotonic gait in pregnant Sprague-Dawley rat” is for females (not male).
References: The list needs to be checked. Dange (1998) is not in the list. Some of the references listed were not cited in the RCD (for example: Bronzon and Jones, 1989).

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Department of Pesticide Regulation

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MEMORANDUM

TO: Tom Moore, Ph.D.
Acting Branch Chief
Medical Toxicology Branch
Dept. of Pesticide Regulation, Cal-EPA

FROM: Andrew Rubin, Ph.D., D.A.B.T.
Staff Toxicologist, Health Assessment Group
Medical Toxicology Branch
Dept. of Pesticide Regulation, Cal-EPA

DATE: June 19, 2014

SUBJECT: RESPONSE TO OEHHA COMMENTS ON THE DRAFT CARBARYL OCCUPATIONAL / BYSTANDER RISK CHARACTERIZATION DOCUMENT

In a memo dated July 22, 2013, OEHHA provided commentary on DPR's carbaryl occupational / bystander risk characterization document (draft of July 12, 2012).

A summary of those remarks appears on pages 1-2 of the OEHHA memo, followed by general comments (pages 2-3), specific comments relating to inhalation toxicity (pages 3-4), dermal toxicity (page 4), acute oral toxicity (pages 4-7), subchronic oral toxicity (page 7), chronic oral toxicity (page 8), genotoxicity (page 8), oncogenicity (pages 8-9), reproductive and developmental toxicity (pages 9-11), developmental neurotoxicity (page 11), pre- and postnatal sensitivity (pages 11-12), aggregate exposure toxicity (pages 12-13), margins of exposure and oncogenic risk (page 13) and editorial comments (pages 13-15). DPR's responses to these comments appear in the following paragraphs. Reference citations appearing herein are listed either in section X. of the revised RCD (dated February xx, 2014) or in OEHHA's original memo.

General Comments (OEHHA critique, pp. 2-3)

1. OEHHA comment—page 3, paragraph 2: "For the endpoints analyzed by BMD in the draft RCD, all BMR were set primarily at 10 percent for both quantal and continuous data. The use of a 10 percent BMR for brain cholinesterase (ChE) inhibition was justified by the lack of overt clinical signs and histopathology, as discussed in a chronic toxicity study review (page 112). While DPR suggested that a lower BMR of 5 percent may be more appropriate for cholinergic signs (page 146), the modeling result using the BMR was not used in the calculation of the MOEs. OEHHA supports the consideration of 5 percent as the BMR for this endpoint. OEHHA typically uses a 5 percent BMR for the dose-response analysis of quantal data (OEHHA, 2008)."

DPR response: We judged the effects of carbaryl to be mild at the lowest dose found to elicit a response. Consequently, we used at 10% BMR to characterize toxicity.

2. OEHHA comment—page 5, paragraph 2: "OEHHA suggests that DPR reconsider the selection of 1 mg/kg-day as the POD because additional data analysis is needed. The calculation of MOEs should be based on the most sensitive endpoint, represented by the lowest POD, which is 0.25 mg/kg-day in this draft of the RCD. PODs need to be determined for the three cited oral gavage studies with only LOELs established, for comparison."

DPR response: Regarding selection of 1 mg/kg-day as the POD, see item #2 under "Specific Comments" below.

Regarding establishment of PODs for the three cited oral gavage studies which generated LOELs, but not NOELs (i.e., Brooks and Broxup, 1995b and 1995c; Brooks et al. 1995): (1) both of the Brooks and Broxup studies contained brain cholinesterase data amenable to BMD analysis (note: the results of such analysis on the 1995b study were discussed in the draft RCD in section IV.A.1.a.); and (2) the Brooks et al. study contained motor activity data that were also a candidate for a BMD approach.

For the brain ChE data in the two Brooks and Broxup studies (1995b and 1995c), datasets for the most sensitive post-dosing time and gender from each study were chosen for analysis. The 1995b study utilized a dose range of 0, 10, 50 and 125 mg/kg. The Hill model for the analysis of continuous data was applied to male whole brain ChE at 0.5 hours post dose, generating an LED_{10} of 0.61 mg/kg and ED_{10} of 0.95 mg/kg (originally run using USEPA BMDS version 2.1, confirmed using USEPA BMDS version 2.3.1). While this value fell slightly below the NOEL of 1 mg/kg selected as the critical acute endpoint, it was a reasonable approximation of that value. This analysis is discussed in the Risk Appraisal section of both the draft and the revised RCD (section V), and the actual output of the BMD application was added to the revised RCD as Appendix II.

For the 1995c study, which utilized a dose range of 0, 10, 30 and 90 mg/kg, the Hill model was used for female hippocampal ChE at 1 hr, generating an LED_{10} of 0.85 mg/kg and ED10 of 3.61 mg/kg (though the modeling may not have been entirely statistically satisfactory for this dataset), again confirming the choice of 1 mg/kg as the critical acute endpoint.

For the study of Brooks et al. (1995), the mean motor activity count data for all six 10-minute intervals for both sexes was selected for BMD analysis, as was the most sensitive individual 10-minute interval. Using the Hill model, the LED_{10} and ED_{10} for mean motor activity counts in males were 3.30 and 6.58 mg/kg, respectively. For females those values were 4.91 and 9.54 mg/kg. In both cases, the model may not have been statistically valid, but the numbers were suggestive of effects at higher than the critical NOEL of 1 mg/kg. The male LED_{10} and ED_{10} for the 11-20 minute interval were 0.93 and 3.76 mg/kg, while the female ED_{10} for the 1-10 minute interval was 9.5 mg/kg (the model could not calculate an LED10). Again, while the model may not have been statistically valid in a technical sense, the analysis offered no reason to change the critical NOEL.

Finally, it should be noted that the dose ranges used for all three of these studies did not go below 10 mg/kg, while the lowest dose for the critical study of Robinson and Broxup (1997) was 1 mg/kg. The fact that only a slight increase in "slight hypotonic gait" may exist at that dose made it clear that 1 mg/kg presented the strongest, most defensible acute POD for regulatory purposes.

Specific Comments (OEHHA critique, pp. 3-13)

1. OEHHA comment—page 4, paragraph 4: "OEHHA concurs with use of the critical subchronic study [Austin, 2002a] to address acute, subchronic and chronic dermal exposure to carbaryl. OEHHA suggests using BMD analysis to better characterize the POD for MOE calculation."

DPR response: Close examination of the brain cholinesterase dataset from this study (see draft RCD Table III-6, copied below for easy reference) reveals that it is not a good candidate for BMD analysis. This is because (1) even when statistically significant, the level of inhibition was relatively small (15% in males [$p<0.05$] and 9% in females [$p>0.05$] at 50 mg/kg/day, the lowest dose showing statistical significance); and (2) partly because of this low level of inhibition, dose responsiveness was not evident. Selection of a traditional NOEL was considered to be the more defensible approach.

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

*p<0.05

2. OEHHA comment—pages 4 (middle) - 7 (middle): OEHHA concluded that the BMD approach to setting a POD for acute toxicity using slight hypotonic gait in the study of Robinson and Broxup (1997)—which in DPR's hands resulted in a LED_{10} of 0.25 mg/kg—was more appropriate than setting the NOEL at 1 mg/kg.

DPR response: We recognize the plausibility of OEHHA's suggestion that 1 mg/kg may not constitute a NOEL, and that the BMD approach may offer an appropriate alternative approach to POD determination. This was manifest in our discussion of the hypotonic gait data in three separate sections of our RCD (the Toxicity Profile, Hazard Identification and Risk Appraisal sections) and in Figure 2 of the RCD, which is copied below. The possibility that the acute MOEs could be overestimated by as much as 4-fold (thus underestimating risk) is clearly stated in at least three places in the revised RCD:

> 1) Section IV.A.1.a. (Risk Assessment, Hazard Identification): "While both critical acute values---the NOEL of 1 mg/kg and the LED_{10} of 0.25 mg/kg---were plausible, this risk assessment used the NOEL of 1 mg/kg to calculate the acute MOEs for inhalation and oral exposure in the Risk Characterization section. This was done in recognition of the clearer experimental support for effects at 10 mg/kg. In no way, however, did this negate the possibility that the most sensitive neurobehavioral endpoints---hypotonic gait and tremors---were slightly induced even at 1 mg/kg. Indeed, use of the resultant 0.25 mg/kg LED_{10} would result in acute MOEs that are 4-fold less than those calculated for the relevant exposure scenarios. MOEs calculated using 1 mg/kg should be viewed with a high degree of seriousness since they could be underestimating the actual acute risk."
>
> 2) Section V.A.1.a. (Risk Appraisal): "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. MOEs calculated using the 1 mg/kg NOEL may thus underestimate the actual risk by a factor of 4."
>
> 3) Section V.B.1. (Risk Appraisal): "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_{10} 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."

Furthermore, the BMD-derived 0.25 mg/kg value was actually used as part of the acute risk evaluation in DPR's dietary risk assessment (DPR, 2010).

Despite the plausibility of the BMD approach, there was reason to question the biological significance of the hypotonic gait incidence data at 1 mg/kg. Points raised in the RCD included the near absence of statistical significance at 1 mg/kg, the strength of the incidence data at 10

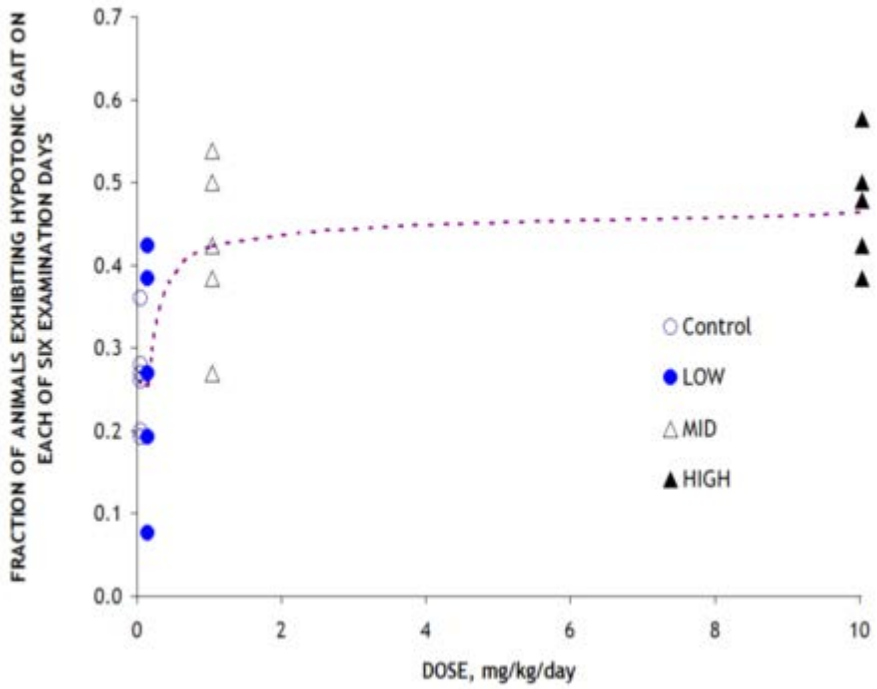
mg/kg both for body weight gain decrements and for several other neurotoxic parameters in addition to hypotonic gait, and the proximity of the 1 mg/kg NOEL to LED_{10}s from the Weinberg (2008) inhalation toxicity study and the Moser (2007) rat cholinesterase study. We have added to the final draft RCD (section IV.1.a.) a further statement to the effect that there was a lack of consistent dose responsiveness throughout the dose range at most time points.

It is also worth reiterating a point made in section V.A.1.a. of the revised RCD, to wit, that the oral gavage exposure regimen was likely to overestimate toxicity by that route compared to the more plausible dietary route:

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.

In conclusion, we view the NOEL approach to acute toxicity taken by the draft RCD to be scientifically valid and defensible. Moreover, our discussion in both the draft and revised RCDs of the BMD approach favored by OEHHA was extensive and provides the reader with a strong sense of its uses and uncertainties.

Figure 2. Scatter plot depicting the fraction of animals exhibiting slight hypotonic gait on each of six examination days[1]; the dotted line is a representation of the average response at the doses tested.



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

3. OEHHA comment—pages 7 (last paragraph) and 8 (paragraph 3): OEHHA recommends use of a BMR of 5% instead of DPR's use of 10% to characterize chronic oral risk.

DPR response: As noted in the draft RCD (page 112) and in the revised RCD (section IV.A.1.c.), the choice of 10% as the BMR was 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. Thus we considered the 20% level of inhibition noted in females at the low dose of 3.7 mg/kg/day to be a relatively mild response, appropriately characterized by the 10% BMR used in the analysis.

4. OEHHA comment—page 8 (penultimate paragraph): OEHHA suggests that the genotoxicity data base be updated.

DPR response: We feel that the current data are more than sufficient to characterize carbaryl's genotoxic potential (a view shared by OEHHA). Newer studies, if they exist, are unlikely to change that.

5. OEHHA comment—page 9, second paragraph: (a) OEHHA discovered an error in the scaling factor used to convert mouse doses into human doses in the Hamada (1993b) oncogenicity study. Thus while a factor of 0.144 was used in the draft RCD, a factor of 0.153, which was expressly calculated in the draft RCD, should have been used. (b) OEHHA also questioned the use of the term "systemic" doses in the description of this study, since these were actually calculated internal doses. (c) OEHHA states that there was no explanation of how the ppm values were converted to dosage terms.

DPR response: (a) We acknowledge the scaling factor error discovered by OEHHA and have corrected the resultant Multistage Cancer Slope value and all of the cancer risk values in the revised RCD. While the error was small and did not affect interpretation of the results, we appreciate the care taken by the OEHHA reviewer which led to this discovery. (b) The term "systemic" has been changed to "internal" at the point in the document mentioned by OEHHA. We agree that "systemic" may not be the correct dosage expression where complete absorption was not verified by experimental data. (c) Dietary ppm values (i.e., mg/kg diet) were calculated from internal doses by the study investigators using the percent carbaryl incorporated into the feed, the rate of food consumption and the body weight. As this was a standard calculation, it required no explanation in the RCD.

6. OEHHA comment—pages 10 (bottom) and 11 (top): "OEHHA supports DPR's consideration of results from the dog studies for POD determination. However, OEHHA suggests that a POD also be determined for increased pup mortality from the Immings et al. (1969) study. This POD would be compared to other PODs of the same exposure route and

duration to insure that the selected POD for MOE calculations would be protective of the reproductive and developmental toxicity from carbaryl exposure."

DPR response: Immings et al. (1968) did indeed establish a developmental LOEL at 2 mg/kg based on based on the non-statistically significant increase in stillbirths at that dose. However, for a host of reasons, we were reluctant to use this study to establish a regulatory endpoint (POD). These included the following:

1. A clear dose-response relationship was not present.

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), which may indicate that the dog is not a good model for humans, particularly in the case of developmental toxicity where mechanisms other than cholinesterase inhibition may be in play.

3. Khera (1976) noted that, unlike other mammals, the dog sheds immature diploid ova, which then undergo a period of maturation and reduction to haploidy before being receptive to sperm. This could generate an altered reproductive sensitivity to xenobiotics in the dog, decreasing its relevancy as a model for potential effects in the human.

4. 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.

5. There was concern that many of the affected pups were conceived during a period of elevated maternal illness.

Even so, we accept that carbaryl may have developmental impacts, as was evident in the extensive discussion of this subject in the draft RCD. In light of OEHHA's comment, and in order to strengthen our mutual concern in this area, we added the following statement to the Risk Appraisal section of the revised RCD (section V.A.1.g.):

> "In conclusion, the studies reviewed for this document suggest that carbaryl has developmental and/or reproductive effects in humans and laboratory animals. Among laboratory animals, dogs appear to be the most sensitive species. In view of the unclear dose-response relation in the study of Immings et al. (1969)---which was also true for Smalley et al. (1968)---it would be difficult to extrapolate the LOEL of 2 mg/kg/day to an estimated NOEL. Nonetheless, it is a reasonable assumption that such a NOEL would be less than the critical acute NOEL of 1 mg/kg and perhaps less than the subchronic / chronic LED10 of 0.5 mg/kg/day. If, for example, the "actual" NOEL approximated the alternative critical acute LED10 of 0.25 mg/kg proposed in DPR's dietary risk characterization (DPR,

2010), then it is clear that individuals of reproductive age and their offspring may not be adequately protected under current use practices."

7. OEHHA comment—page 11, paragraphs 2 and 3: OEHHA advocates adding changes in brain morphometry in F1 pups and adults to the toxic signs noted at 10 mg/kg/day in the developmental toxicity study of Robinson and Broxup (1997). They assert that "the bilateral decrease in the size of the forebrain (line A) in the first generation (F1) male adults and the bilateral decrease in cerebellar length (line F) in F1 female pups are indicative of adverse effects of carbaryl on brain development".

DPR response: While it is possible that the observed brain morphometric changes in F1 pups and F1 adults were caused by carbaryl, we view the cited data as both insufficiently robust and plausibly due to random variation, making them poor candidates for listing as LOEL determinants. More extensive measurements were called for. Nonetheless, we have strengthened the statements in the Toxicity Profile section of the revised RCD to call the reader's attention to the morphometric changes, as follows:

"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, as well as certain changes in brain morphometric measurements at that dose, may have resulted from carbaryl exposure."

8. OEHHA comment—page 12, paragraph 1: "OEHHA remains concerned that the PODs selected to calculate the MOEs may not be protective against the fetal mortality observed in the dog study (Immings et al. (1969)). If a POD cannot be determined from the reported data in Immings et al. (1969), then an additional uncertainty factor may need to be considered to account for the fact that the study only established a LOEL."

DPR response: DPR's treatment of the study of Immings et al. (1969) is summarized above in the response to comment #7. We are satisfied that the uncertainties surrounding carbaryl's potential developmental impacts have been sufficiently addressed in the revised RCD.

9. OEHHA comment—page 12, paragraph 3: "...rationale should be provided as to why dietary exposure was not considered in the aggregate exposure for adult bystanders, when this human receptor is used to represent the general public."

DPR response: Table IV-11 in the RCD (draft and revised), entitled "Non-oncogenic and oncogenic risks resulting from inhalational carbaryl exposure to bystanders – agricultural and public pest control applications" indeed does express the aggregate risk to adult and infant bystanders resulting from inhalation and dietary exposure.

10. OEHHA comment—page 12, paragraph 5: OEHHA recommends adding a dietary component to the oncogenic aggregate risk values for handlers (Table IV-7a) and re-entry workers (Table IV-7b).

DPR response: As suggested by OEHHA, we have added the Western USA dietary risk established in the dietary risk assessment (DPR, 2010), 3.68×10^{-6}, to the aggregate risk calculations for handlers and re-entry workers in the revised RCD.

11. OEHHA comment—page 13, paragraph 3: "For cancer risk, DPR calculated lifetime exposures only for workers (dermal and inhalation routes) and for adult bystanders (inhalation route). DPR used a probability of one in a million for evaluating cancer risk. OEHHA concurs with the use of this level to evaluate cancer risk. However, OEHHA recommends that cancer risk should be calculated for the general population exposed to carbaryl in the ambient air. While ambient air concentration was not calculated in the EAD, the EAD indicated that the bystander exposure level could be used as an upper bound for an ambient air exposure level. OEHHA recommends that the lifetime exposure to carbaryl should include vulnerable periods such as third trimester in utero and early childhood. For this reason, OEHHA suggests the use of age-specific inhalation rates and ASFs (OEHHA, 2009b) in the calculation of lifetime inhalation exposure and cancer risk, respectively."

DPR response: OEHHA correctly states that, in the absence of ambient exposure estimates, we used bystander exposures to estimate an upper bound ambient oncogenic risk. Their further argument that age sensitivity factors (ASFs) may be necessary for a fully refined estimate of oncogenic risk merits consideration. We referred to the OEHHA's citation to calculate oncogenic risk in light of default ASFs for exposures during the third-trimester-to-age-2 period and during the age-2-year-to-age-16-year period. These are now reported in section IV.C.6. and discussed further in section V.A.2. as follows:

> "It is important to note that the estimations of oncogenic potency for various exposure categories (mostly occupational, but also bystander) do not take into account the possibility that early lifestages may be more oncogenically vulnerable than mature stages. In a lengthy discussion of this issue, OEHHA (2009b) concluded that, in the absence of specific data, it may be appropriate to apply default age sensitivity factors (ASFs) to arrive at more refined oncogenicity estimates. While such considerations may not apply to occupational exposure scenarios, we calculated oncogenic risk to bystanders in footnote 18 using the default early lifestage ASFs developed in that document. Thus the oncogenic risk estimate for adult bystanders rose from 1.81×10^{-6} to 3.06×10^{-6} (or from 5.49×10^{-6} to 6.78×10^{-6} if dietary exposure is included) when possible early lifestage vulnerability was taken into account. Early lifestage exposure in the absence of lifetime exposure may also be associated with significant oncogenic risk. Even so, we are not aware that studies designed to evaluate lifestage vulnerability exist for carbaryl."

Editorial comments (OEHHA critique, pp. 13 - 15)

1. OEHHA comment—page 13: "A list of tables would be helpful for the readers."

DPR response: A table listing capability is not present in the WordPerfect 12 version that was used to assemble this risk characterization document.

2. OEHHA comment—page 13: OEHHA suggests making the lists of exposure scenarios addressed in the draft RCD consistent each time they are mentioned. For example, the title page mentions only occupational and bystander exposure scenarios, while at other places in the document occupational, bystander, ambient and/or dietary scenarios are mentioned.

DPR response: Since the assessment deals overwhelmingly with risks associated with occupational and bystander exposures, the title page reference is appropriate as written. Where necessary within the document, ambient scenarios are mentioned in order to call the reader's attention to them, though they play little role in the overall assessment. The instance in the draft RCD where dietary scenarios are mentioned was clearly incorrect, essentially a holdover from the dietary assessment document on carbaryl. It has been removed from the revised RCD.

3. OEHHA comment—pages 13-14: "Summary and Hazard Identification sections: It would be helpful to have a table summarizing the critical studies, PODs and endpoints used for the MOE and oncogenic risk calculations in this section instead of at the end of the document (Table VII-1, page 164) as part of the reference dose and concentration presentation."

DPR response: The following statement has been added to the beginning of the Hazard Identification section (section IV.A.): "All critical NOELs and LEDs identified in the following sections are listed in Table VII-1 at the end of this document."

4. OEHHA comment—page 14: "Section III, Toxicology Profile: The summary table for acute toxicity is missing."

DPR response: A summary of acute toxicity studies has been added to the revised RCD at the end of section III.B.

5. OEHHA comment—page 14: "Page 42, Paragraph 1: According to paragraph 1, 'Subchronic NOELs and LOELs are summarized in Table III-8.' These values are actually summarized in Table III-10.

DPR response: Corrected.

6. OEHHA comment—page 14: "Page 43: The data in Moser (2007) have been published in Toxicological Sciences 114(1):113-123 (2010). The results, if necessary, and the citation in the RCD should be updated."

DPR response: The open literature citation has been added to the revised RCD in three separate places. The difference reported in the article for brain ChE inhibition LED10—1.14 mg/kg in the analysis of Setzer, 1.3 mg/kg in the cited article—is minor and did not require comment in the revised RCD.

7. OEHHA comment—page 14: "Pages 45 and 69: According to the text, the systemic NOEL for the subchronic dermal Austin (2002a) study is 20 mg/kg-day. However, Table III-10 lists the systemic NOEL as 100 mg/kg-day. This inconsistency should be addressed."

DPR response: ChE inhibition formed the basis of the systemic NOEL determination for this study, which was not clear in Table III-10, as pointed out by OEHHA. This discrepancy is now corrected in the revised RCD.

8. OEHHA comment—page 14: "Page 48, Paragraph 1: According to paragraph 1, 'Chronic NOELs and LOELs are summarized in Table III-9.' These values are actually summarized in Table III-10."

DPR response: Corrected.

9. OEHHA comment—page 14: "Page 87, Paragraph 6: Space should be provided between the title for Table III-13, 'NOEL and LOEL values in laboratory animal studies on the reproductive toxicity of carbaryl' and the statement above so that the title appears on the top of the table"

DPR response: Corrected.

10. OEHHA comment—page 14: "Page 95: In the table, superscripts 3 and 4 should be c and d."

DPR response: Corrected.

11. OEHHA comment—page 14: [Page 98, paragraph 3] Add the subtitle "Conclusions" to the paragraph just following the section entitled "F1 adults".

DPR response: Done.

12. OEHHA comment—page 14: "Pages 99-100 in Table III-16a: There seems to be an error in the incidence for 'Signs' for the gd 20–1.0 mg/kg/group. It is lower (10/26) than that (11/26) for hypotonic gait alone. The incidence of 'Signs' should be the same or higher since it represents the number of animals with one or more signs."

DPR response: Error noted and corrected in what is, in the revised draft, Table III-17a. In addition, further information was added to footnote "d" to clarify how these cumulative data (i.e., 'Signs [per animal basis]' were collected. The footnote now reads:

"Signs (per animal basis) was an aggregate indicator of carbaryl effects noted in the FOB tests. They were enumerated by the risk assessor. Animals for which there were one or more signs were counted only once per test day. Only positive signs were considered (i.e., the decline in incidence of moderate dilation of pupils was not included). Changes in defecation or urination were not included since they did not bear a clear relation to dose."

13. OEHHA comment—page 14: "Page 100, footnote d: The incidences appeared to be for animals with one or more signs, not only those with 'more than one sign' as indicated in this footnote."

DPR response: Error noted and corrected (see revision of footnote "d" in item #12 above).

14. OEHHA comment—page 15: "Page 111, Paragraph 3, line 2: Hamada reference should be 'Hamada 1987' instead of 'Hamada 1997'."

DPR response: Error noted and corrected.

15. OEHHA comment—page 15: [Page 116, second paragraph] OEHHA recommends changing the description of the process used to arrive at the benchmark dose model ultimately used to generate the cancer potency factor. In particular, reference to the extensive analysis done for DPR's dietary risk assessment on carbaryl (DPR, 2010), which tested many of the benchmark dose models available in USEPA's BMD software, should be removed from the revised RCD because the "multistage cancer model is the only appropriate model for the determination of potency".

DPR response: We concur with this view. Accordingly, the cited passages in the Hazard Identification section of the revised RCD have been rearranged to more accurately reflect the nature of the BMD analysis ultimately used for this risk assessment. We have also updated the reference to the particular BMD software version used in the revision (version 1.10).

16. OEHHA comment—page 15: "Page 117: Paragraph 2, line 2: Typographical error, 'adquate' should be 'adequate'."

DPR response: Error noted and corrected.

17. OEHHA comment—page 15: "Page 131, Paragraph 1, line 2: This line reads, 'hazard index approach (footnote c, Table xxxg).' Please clarify which table is being referred to by Table xxxg. Also, location of Table xxxh on line 11 needs to be identified.

DPR response: Errors noted and corrected.

18. OEHHA comment—page 15: "Pages 131-139: Mislabeling of the tables: xxxg, xxxh, xxxc."

DPR response: Errors noted and corrected.

19. OEHHA comment—page 15: "Page 134, footnote c: The equation used to calculate the aggregate MOE is referred to as 'Total MOE', not 'Hazard Index' as written in the footnote."

DPR response: The reference to "hazard index" in footnote "c", Table IV-7a (p. 134 of the draft RCD), is appropriate. However, the word "aggregate" has been substituted for "combined" in the revised RCD.

20. OEHHA comment—page 15: "Page 134, footnote d: Aggregate oncogenic risk was the 'sum', not 'product' as written in footnote."

DPR response: Error noted and corrected.

21. OEHHA comment—page 15: "Page 158, Paragraph 4, line 3: Which table is being referred to by 'Table xxxl'?"

DPR response: This particular reference is to Table IV-11. All table designations erroneously containing "xxx" identifiers (used during the drafting process) have been corrected in the revised RCD.

22. OEHHA comment—page 15: "Page 162, Footnote 18: the units are wrong: 'm3/mg/hr' should be 'm3/kg/hr."

DPR response: Error noted and corrected.

23. OEHHA comment—page 15: "Page 179 (Appendix I): the description below the Probit Model stated, 'Slight hypotonic gait data in males (top dose deleted)'. This note appears to be a typographical error since the title of this Appendix I, 'Benchmark dose extrapolation for induction of slight hypotonic gait in pregnant Sprague-Dawley rat' is for females (not male)."

DPR response: Error noted and corrected.

24. OEHHA comment—page 15: "References: The list needs to be checked. Dange (1998) is not in the list. Some of the references listed were not cited in the RCD (for example: Bronzon and Jones, 1989)."

DPR response: The Dange (1998) citation has been added to the reference list in the revised RCD. Bronzan and Jones (1989), which was on the reference list of the draft RCD, has been removed from the revised RCD.

Office of Environmental Health Hazard Assessment

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Matthew Rodriquez
Secretary for
Environmental Protection

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MEMORANDUM

TO: Lisa Ross, Ph.D., Chief
Worker Health and Safety Branch
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P.O. Box 4015
Sacramento, California 95812-4015

FROM: Anna M. Fan, Ph.D., Chief *(Original Signed By Dr. Anna Fan)*
Pesticide and Environmental Toxicology Branch
1515 Clay Street, 16th Floor
Oakland, California 94612

DATE: July 25, 2013

SUBJECT: COMMENTS ON THE DRAFT EXPOSURE ASSESSMENT DOCUMENT FOR CARBARYL

The Office of Environmental Health Hazard Assessment (OEHHA) has reviewed the draft Exposure Assessment Document (EAD) for occupational and ambient air exposure to carbaryl, prepared by the Department of Pesticide Regulation (DPR), dated June 28, 2012. Our comments are provided in the attachment. OEHHA has provided comments in a separate memorandum on the Risk Characterization Document for Carbaryl. OEHHA reviews risk assessments prepared by DPR under the authority of Food and Agriculture Code section 11454.1.

OEHHA has provided a number of comments on the exposure assessment methodology and conclusions of the draft EAD. These comments and our recommendations, as well as suggested clarifications, additions and corrections, are contained in the attachment.

Thank you for providing this draft document for our review. If you have any questions regarding OEHHA's comments, please contact Dr. Charles Salocks at (916) 323-2605 or me at (510) 622-3200.

Attachment

cc: Charles B. Salocks, Ph.D., D.A.B.T.
Chief, Pesticide Epidemiology Section
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment

OEHHA's Comments on the Draft
Exposure Assessment Document for Carbaryl

The Office of Environmental Health Hazard Assessment (OEHHA) is responding to a request from the Department of Pesticide Regulation (DPR) to comment on the draft Exposure Assessment Document (EAD) for carbaryl [*1*-naphthyl *N*-methylcarbamate]. OEHHA reviews risk assessments prepared by DPR under the authority of Food and Agricultural Code Section 11454.1, which requires OEHHA to conduct scientific peer reviews of risk assessments conducted by DPR.

SUMMARY

The EAD was comprehensive and assessed a wide range of exposure scenarios of workers in agricultural and non-agricultural settings, bystanders and residents. The uncertainties and calculations were well described.

OEHHA concurs with most of the approaches and factors used in the EAD, but have the following suggestions:

- Update exposure calculations using the current U.S. Environmental Protection Agency (US EPA) guidelines for dislodgeable foliar residues (DFR) and consider US EPA assumptions for clothing of residential handlers and resident reentry level. Update breathing rate defaults or consider OEHHA's breathing rates to calculate exposure.
- Consider providing exposure estimates from take-home residues and estimates of ingestion doses for children with pica.
- Provide more detailed descriptions of the monitoring studies, and provide justification for selecting certain surrogate chemical studies and rejecting others.
- Reconsider clothing assumptions used in the homeowners' exposure scenario, and calculate post-application exposure without re-entry interval for residential users.
- Identify representative activities within the high use season instead of establishing representative activities independently of the pesticide use.

In addition, we offer some suggestions to improve the readability of the document. These suggestions include revising the abstract to include key issues beyond risk assessment conclusions, expanding the introduction to orient the reader, and adding conclusion statements for each major section.

SPECIFIC COMMENTS

ABSTRACT (pages 7 and 8)

The abstract summarizes the key conclusions regarding risk and is not actually an abstract. Listed scenarios were identified as "having the highest level of concern" based on comparison of exposure to toxicity profile (as stated on page 88 in the RISK APPRAISAL section). OEHHA recommends that if the section continues to be named as an "abstract" it should be revised to reflect the key points from each of the sections in the EAD (environmental concentrations, pharmacokinetics, scenarios, exposure assessment…), not solely conclusions regarding risk.

INTRODUCTION (page 8)

The introduction contains only two sentences on carbaryl's use and a statement about its mode of action. OEHHA recommends this section be revised to provide the reader with information on the layout and content in the EAD.

EXPOSURE SCENARIOS (pages 17 to 27)

The EAD describes a wide range of occupational and non-occupational scenarios including application exposure of handlers, and post-application exposure of workers, bystanders, residents (including children at play on treated lawns), and swimmers. For the exposure of workers entering a treated area (reentry) after a certain time (i.e., the reentry interval or REI), DPR used a tiered approach to select work activities. Since carbaryl is used on many crops, some of the crops were grouped and portrayed by a representative crop. For each representative crop, work activities with the highest potential for exposure were selected as scenarios for exposure calculations.

OEHHA concurs with the choice of scenarios in the EAD. However, OEHHA recommends that the EAD includes exposure to carbaryl in take-home dust. There is evidence in recent studies showing that non-volatile pesticides can be found in significant amounts in homes of agricultural workers (Bradman et al. 2007, Curwin et al. 2007, Golla et al. 2012, Gunier et al. 2011) or homes neighboring agricultural fields (Gunier et al. 2011). Gunier et al. (2011) investigated the association between proximity to fields and pesticides residues in carpet dust. Significant associations were determined for simazine, phosmet, chlorpyrifos, iprodione and chlothal-dimethyl. While the study did not find a significant association for carbaryl, there were limitations to the study, including small sample size and lack of information about pesticide use patterns. In addition, the EAD should note the possibility that there are potentially multiple sources of carbaryl contributing to the levels detected in indoor dust and that an assessment of aggregate exposure may be warranted.

PHARMACOKINETICS (pages 24 to 31)

The dermal absorption was set at 70 percent (%) based on results from a dermal absorption study in rats following 12 hours of exposure to 4 microgram per square centimeter (µg/cm^2) of carbaryl. This value was based on the "best available data for the anticipated range of occupational and residential exposures" (page 89). DPR decided that the selected study, which used acetone as a solvent vehicle, was acceptable because comparison of two other studies showed that acetone did not alter (increase) the dermal absorption of carbaryl (page 26 and EXPOSURE APPRAISAL, *Estimated Dermal Absorption*, page 89). Other *in vivo* data from studies in rats were available but their doses were high (35.6 µg/cm^2) compared to anticipated human carbaryl exposures, and this may have resulted in underestimation of percutaneous absorption because high skin surface loading typically results in lower absorption efficiencies.

Human data were not used because the study was not well conducted (pages 30 and 31). All *in vitro* data available were derived from studies that used high doses (40 µg/cm^2), and consequently the percutaneous absorption rate may have been underestimated. Results from the *in vitro* studies (which showed 20-40% absorption) were within the range of the rates observed with the high-dose rat study. The RISK APPRAISAL section (pages 88-89) explained clearly how DPR used *in vitro* data in combination with other information as weight of evidence. The study selection was justified and the quality assurance was verified. The dermal absorption rate identified in the EAD was much higher than the rate determined by US EPA, in part because US EPA excluded bound skin residues in estimating penetration (US EPA 2008). OEHHA concurs with the choice of 70 percent for dermal absorption rate.

The EAD used a default value of 100 percent for inhalation intake and uptake since no experimental data were available, and suggested (page 94) that using this default value for the inhalation absorption rate might overestimate exposure. Nevertheless, OEHHA believes the default value is appropriate given the lack of data to support an alternative absorption rate.

Numerous biomonitoring studies were available for determining the pharmacokinetics of carbaryl but the data did not correlate specific metabolites with exposure levels. OEHHA agrees with the conclusion in the EAD that the results of these studies were not usable for the exposure assessment.

ENVIRONMENTAL CONCENTRATIONS (pages 31 to 46)

The EAD provided summaries and evaluations of numerous studies to determine the concentrations (on treated surfaces, water, and air) to be used for the calculation of exposure.

OEHHA suggests that, at the end of each subsection, the EAD include a brief statement of conclusion indicating which specific studies and environmental concentrations will be used to calculate exposures. For example, under ***Water*** (pages 42 to 46), the last paragraph in the section stated that "Reported concentrations of carbaryl in surface water were used in calculating swimmer exposure estimates" (page 43), but no specific study was cited. The water concentration that was used to estimate swimmer exposure appeared as a part of the study description for Walters et al. (2003) (page 45) under the subsection of "Surface Water Monitoring: Application Site."

Dislodgeable Foliar Residues **(pages 31 to 37)**

DPR followed the acceptability criteria set by US EPA (1996) to select the studies for estimating a worker's dermal exposure to DFR. The DFR studies conducted with carbaryl included dissipation studies on field crops, vegetables and fruits, and spot sampling studies. In the absence of carbaryl-specific DFRs for certain crops, DFRs from other chemicals or other crops were used as surrogates. Interpolation of DFR data for the days that were not sampled post-application was done by log-linear regression of data for sampled days; the rationale on the use of this regression was provided on page 91. The transfer coefficients (TC) were considered not chemical-specific but rather crop- and activity-specific. When data were not available, DPR used default TC values from similar cases (that is, a combination of similar activity and a similar crop).

OEHHA concurs with DPR's methodology and DFR and TC values, as summarized in Table 16 (page 36). However, the studies and values may need to be updated using the current version of the US EPA guidelines (2012).

Transferable Turf Residues (TTRs) were determined to estimate the dermal exposure to carbaryl on treated turf and sod (pages 35 to 37). Carbaryl residues were measured in two studies, one using a liquid formulation and one using a granular formulation. On page 35, the introductory sentence to this section stated "Available data do not appear to support a consistent relationship between TTR and exposure." It isn't clear whether this is a general statement that applies to all pesticides or a statement that applies specifically to the TTR data that are available for carbaryl. In the summaries of the two studies, there was no explanation why these results were rejected and surrogate exposure monitoring data were used instead (page 37, first paragraph). OEHHA recommends additional explanation for rejection of the TTR studies. Furthermore, this section of the EAD should indicate why surrogate exposure data were used.

***Air* (pages 37 to 42)**

Ambient Air

The EAD described two studies, conducted by the California Air Resources Board (ARB) and U.S. Geological Survey (USGS), to determine ambient air levels at both urban and rural sites (page 37). The USGS monitored ambient air concentrations of carbaryl in Sacramento County (Majewski and Baston 2002) and carbaryl was detected at both urban and rural sites. The highest concentration was 0.0306 microgram per cubic meters (µg/m^3). Results of this study suggested that the general population can be exposed to airborne carbaryl in areas that are distant from application sites.

Ambient air monitoring was conducted by ARB in three counties with relatively high carbaryl use (Fresno, Tulare, and Kings) during times when peak use was anticipated (ARB 2008). Carbaryl was not detected in any of the samples. The EAD did not use the results from these studies to estimate exposure to carbaryl in ambient air. Instead, a higher air concentration (estimated for bystanders at the application site) was selected to provide a "health-protective" estimate (page 88). These air concentrations ranged from 43.9 µg/m^3 for a one-hour exposure to 1.59 µg/m^3 for chronic and lifetime exposures, as shown in Table 35. OEHHA concurs with the selection of air concentrations for bystander exposure to calculate ambient air exposure.

Application Site Air Monitoring: Agricultural Applications

Data from two air monitoring studies for carbaryl associated with agricultural applications were available, but were determined not acceptable because of limited sampling and lack of information about application and monitoring conditions (page 37). Instead, data from a methyl parathion airblast application (Barry 2006) was used as a surrogate to determine the air concentration for bystander exposure (pages 37 to 40). The justification was that airborne concentrations and drift depend on the equipment, timing, and location of the application, and the vapor pressure of the active ingredient rather than the chemical structure of the active ingredient (page 38). OEHHA considers the justification reasonable. However, the potential impact of a 10-fold higher vapor pressure for methyl parathion (page 38, 2nd paragraph) compared to carbaryl on the inhalation exposure estimates for bystanders should be discussed in the EAD.

Application Site Air Monitoring: Applications in Urban Areas

Multiple studies were available that monitored on-site air in California (Table 18, page 41). The highest concentration detected in any of these studies was 12 µg/m^3 (Neher et al. 1982). Two studies were available that monitored off-site air in California (Table 19), one monitoring drift from an aerial application and one monitoring off-site air following a mist blower application. The conclusion of the study summaries provided no indication which one was selected as a basis for estimating bystander exposure from applications in urban areas. This information was provided much later on page 87 (Table 87). OEHHA suggests that the EAD include a discussion on the merits of the Neher et al.

(1982) study and provide justification why it was used as a basis for calculating these exposure estimates.

***Water* (pages 42 to 46)**

Carbaryl has been detected in surface water and ground water as a result of rainwater, and runoff from application sites. Reported concentrations of carbaryl in surface water were used in calculating swimmer exposure estimates. Walters *et al*. (2003) measured the highest carbaryl concentration in a swimmable body of water at 6.94 microgram per liter (µg/liter) in a fishpond in Sacramento County; this concentration was used to estimate swimmer exposure. OEHHA agrees with the selection of the surface water data that were used to calculate swimmer exposure.

EXPOSURE ASSESSMENT (pages 46 to 88)

Exposures (as absorbed dosages) of workers, bystanders, residents, and swimmers were calculated using the selected environmental concentrations and other parameters such as exposure rates, protective clothing factors, body weights, skin surface area, absorption factors, and exposure durations. The durations included short-term, intermediate-term, and long-term (annual and lifetime) exposures.

***Occupational Handler Exposure* (pages 47 to 66)**

The dermal and inhalation exposures of handlers (mixer, loader, and applicator, abbreviated M/L/A) under agricultural and non-agricultural settings (e.g., lawns, golf courses and rights of way) were determined for each application method (such as hand-held, airblast, groundbloom, aerial, and ground applications). They were calculated using environmental concentrations, exposure rates from carbaryl or surrogate monitoring studies or the US EPA Pesticide Handler Exposure Database (PHED), applicable protection factors, and various assumptions. Short-term exposure estimates were calculated using application sizes (acres treated) recommended by US EPA as realistic maxima. The application rate was the maximum allowed per the product label.

Seasonal, annual and lifetime exposures were also estimated for all handlers, except those applying carbaryl on rights-of-way because repeated exposures are not anticipated for this scenario. The application rate was based on the highest annual mean values in California during a 5-year interval. The application size used was the average from the Pesticide Use Report (PUR) data or the typical application size assumed by US EPA. To determine the high-use period, temporal patterns (percent of annual use based on pounds applied per month 2006-2010) were investigated using data from the county that had the highest application rate and seasonal application for a

specific crop. OEHHA concurs with the values for application size and rate, and the high use period.

Monitoring studies and PHED were used to determine exposure rates (µg carbaryl exposure/pounds handled), as discussed under the following two subsections.

Exposure Monitoring Studies (pages 47 to 50, Tables 20-29)
Six studies monitored applicators using hand-held spray equipment. With the exception of Merricks (1997), these studies could not be used to estimate exposure because of problems with the protocol or analysis (page 47). Merricks (1997) monitored exposure of residential handlers applying multiple carbaryl products (page 47, Table 20): a dust product, and liquid products using three liquid application methods (ready-to-use trigger sprayer, hose-end sprayer, and hand-pump sprayer), each involving monitoring of 40 replicates. In this study, all handlers wore gloves, except those using dusters. A glove protection factor (90%) was added to the duster handler exposure since the current label requires the use of gloves for dust products. OEHHA concurs with the use of data from this chemical-specific study to estimate exposure for low pressure handwand M/L/A (page 62, Tables 26 and 27), trigger sprayer and hose-end sprayer M/L/A (page 63, Tables 26 and 27) and dust M/L/A (page 66; Tables 28 and 29). OEHHA also agrees with the glove protection factor and with DPR's statement that the protection factor might underestimate exposure (pages 90-91).

Three studies monitored applicators using airblast equipment. With the exception of Smith (2005), these studies could not be used to estimate exposure as explained on pages 47 to 48. Smith (2005) monitored dermal and inhalation exposure of airblast applicators driving open-cab tractors to carbaryl and wearing either Sou'wester rain hats (15 replicates) or hooded rain jackets (10 replicates). OEHHA concurs with the use of data from this chemical-specific study to estimate exposure for airblast applicator (pages 48-49, 55-58; Tables 21, 24, and 25).

No chemical-specific monitoring data were available for granular applications using a push-type lawn spreader (page 49). DPR used a "well-conducted" surrogate study by Klonne and Honeycutt (1999) with applicators using Dacthal® granular herbicide, containing 0.9 percent dimethyl tetrachloroterephthalate (the active ingredient). Dermal and inhalation exposures were estimated for push type-spreader loaders and applicators (pages 65-66, Tables 20, 28 and 29). OEHHA concurs with the use of a surrogate study to estimate exposure when no chemical specific data was available, but justification for the selection of the study conducted by Klonne and Honeycutt (1999) is needed.

Exposure Estimates Using the Pesticide Handler Exposure Database (page 50, Tables 22-29)

DPR used the PHED to determine exposure estimates for all other handlers (page 50) utilizing the following application methods: aerial (pages 50-53, Tables 22-23), airblast (mixer and loader; pages 48-49, 55-58; Tables 21, 24, and 25), groundboom (pages 58-59, Tables 24 and 25), chemigation (page 59), rights-of-way (page 60, Table 26), backpack sprayer (page 60, Tables 26 and 27), high pressure handwand (page 62, Tables 26 and 27) and broadcast spreader (page 63, Tables 28 and 29).

As discussed in the appraisal (page 89), the most recent studies cited in PHED were conducted in 1994. The measurements done using older equipment and practices tended to overestimate exposure (Beauvais et al. 2007). Because of the degree of uncertainty in the PHED data, DPR used the 90 percent upper confidence limit of the 95th percentile values for short term exposure, and the 90 percent upper confidence limit of the arithmetic mean for long term exposure estimates. Total exposure was assumed to be lognormally distributed with a coefficient of variation of 100% (Beauvais et al. 2007). PHED values were adjusted to sample size by using multipliers. Because no chemical specific data were available, OEHHA concurs with the use of PHED to estimate exposures for these groups of handlers.

***Occupational Post-Application Exposure* (pages 67 to 79)**

For post-application exposure estimates, DPR determined crops where carbaryl is used, as reported in DPR's Pesticide Use Report, selected work activities that represent typical fieldworker activities for a crop group, and identified the activities with highest potential exposures (page 20, Tables 7 and 8). The studies used to calculate the DFR and TC were discussed on pages 31 to 37.

OEHHA suggests that the identification of representative activities included consideration of the extent of carbaryl use. High exposure could occur during high use season. For example, pruning was identified as the representative activity for the use of carbaryl on olive trees (page 22), but according to PUR data (page 75) this activity does not occur during the months of high carbaryl use (July-August). This could have underestimated the exposure.

The EAD identified five studies (Tables 30-31) to estimate post-application exposure: (Klonne et al. 2001a) for olive pruning; (Klonne et al. 2001b) for cabbage scouting, cucumber scouting, and tobacco hand harvesting; (Klonne et al. 2001c) for apple hand thinning; (Klonne and Merricks 2000) for citrus pruning; and (Zweig et al. 1984) for strawberry scouting. Apple DFR data were used as a surrogate for asparagus hand harvesting, lettuce scouting, and corn detasseling. Strawberry DFR data were used as a surrogate for grape leaf pulling, bean scouting, blackberry pruning, potato scouting,

and tomato staking/tying. Citrus DFR data were used as a surrogate for ornamental plant hand harvesting. OEHHA concurs with DPR's choice of selected studies and surrogate data.

Short term post-application exposure estimates were calculated for workers for use of carbaryl on asparagus, beans, blackberry, cabbage and tobacco. Short term and long term post-application occupational exposure estimates were calculated for use of carbaryl on citrus, corn, cucumber, grape, lettuce, olive, ornamental plants, potato, strawberry, and tomato. OEHHA supports the selection methodology of activities and durations of exposure.

Turf maintenance

For workers on turf or sod after application of carbaryl, dermal exposure was assumed to occur the same day as the application because the product label did not specify a post-harvest interval (PHI) for applications to golf courses, lawns, and other turf (page 78). Results from a study of adults doing exercise on oxadiazon-treated carpet (Rosenheck and Sanchez 1995) were used as surrogate data for carbaryl to determine the exposure rate (micrograms per kilogram per hour, µg/kg-hour). Dermal exposure was adjusted using a 90 percent protection factor for covered body regions provided by long sleeves, long pants and shoes. In this scenario, the EAD only determined short term exposure because carbaryl application on turf was infrequently reported (DPR 2012).

OEHHA concurs with these assumptions although the choice of oxadiazon as a surrogate needs to be justified. This study was briefly described, using the same text, on pages 79 and 82, but no details were provided on how the exposure rate was determined. Such explanation should be included as part of the presentation of studies under Transferable Turf Residues (TTR) on pages 35 to 36.

***Residential Handler Exposure* (pages 79 to 81)**

For residential handlers, a surrogate study with Dacthal® (Klonne and Honeycutt 1999) for push-type spreader application and PHED data for backpack sprayer application were used to determine the exposure rates. Data from carbaryl studies (Merricks 1997) were used for workers using handwand, trigger sprayer, hose-end sprayer, and dust can application. The exposure rate determinations assumed that all handlers (push type spreader included) wore protective clothing and chemical-resistant gloves (Table 32).

OEHHA is concerned that this assumption may not be valid based on the following information. On page 17, the EAD stated "In contrast [to liquid formulations], most labels on granular products and baits have user safety recommendations rather than requirements for residential users. As users can legally choose not to follow the

recommendations, exposure estimates for residential handlers of these products do not assume that protective clothing or PPE are used." Page 92 of the RISK APPRAISAL section included the following statement: "Users of pesticides are legally required to follow use directions given on pesticide labels but non-occupational pesticide handlers are not inspected for safety. In recognition of this enforcement gap, exposure estimates were calculated for users not complying with product label requirements for PPE." Furthermore, there was a large difference when exposure estimates were calculated for users NOT complying with product label requirements for Personal Protective Equipment (PPE) (Table 38). When results were compared, exposures without PPE were estimated to be 3 to 80 times higher than those with PPE (page 93). Despite the acknowledgement of a low level of enforcement, DPR still assumed PPE use in the exposure estimate calculations. OEHHA suggests the EAD present only the exposure estimates that were calculated assuming no PPE, as shown in Table 38 (page 92), with handlers wearing loose-fitting shorts and no gloves. US EPA considered that residential applicators would wear short pants, T-shirts and shoes (US EPA 1992, 2012) as a "worse case but common scenario".

Carbaryl liquid applications were restricted to spot treatments of 1000 square feet and 2-4 applications/year at least 7 days apart. The maximum rate allowed was a function of the application type. Carbaryl granular/bait applications were restricted to treatments of 0.5 acre at 8.28 pounds of active ingredient per acre (lb AI/acre) with no minimum reapplication interval or applications/year. Seasonal, annual and lifetime uses were not anticipated. Only the short-term absorbed daily dose (STADD) was calculated (page 80, Table 32). OEHHA concurs with the assumptions and the calculation only for short-term exposure.

***Residential Post-Application Exposure* (pages 81 to 84)**

<u>Dermal Exposure from Reentry onto Treated Lawns (pages 81 to 83)</u>
The representative reentry scenario in residential settings is dermal exposure from contact with treated lawns. Dermal exposure rates for toddlers (3-year olds) and adults on treated lawns were calculated using data from a surrogate study with adults exposed to oxadiazon (Rosenheck and Sanchez, 1995). Adults and toddlers were expected to spend 2 hours per day on treated turf (US EPA 1997).

OEHHA concurs with the assumptions that were incorporated into these calculations as shown on Table 33 (page 82), but suggests that DPR clarifies the assumptions regarding the clothing worn by children and adults. Previous comments about the oxadiazon study also apply to this section.

On page 78, DPR stated that reentry was assumed to occur on the same day as the application without any reentry interval (REI). But the study used to estimate exposure (Beauvais 2012) followed the label requirement "until the spray has dried". Depending

on the weather conditions at the time of application, the drying time could last several hours, particularly along coastal areas of California.

If the EAD used data from a study with REI without adjusting the result to account for a "no REI" assumption, it most likely underestimated exposure. Moreover, OEHHA notes that the assumption "until the spray has dried" is subjective and vague, adding uncertainties to the assessment. US EPA (2012) considered post-application exposure for residential users without re-entry interval. OEHHA suggests that DPR follow US EPA guidance and adjust the data so they are consistent with the EAD assumptions. In addition, the EAD should provide the data and calculations from the Beauvais (2012) study to show how the exposure rates were determined, instead of simply citing this internal report.

Incidental Non-Dietary Ingestion of Pesticides Applied to Turf

Incidental non-dietary ingestion by toddlers of pesticides applied on turf was included to account for hand-to-mouth transfer, object-to-mouth transfer and soil Ingestion (pages 83-84). A carbaryl-specific non-dietary ingestion exposure monitoring study was not available. Consequently, exposure estimates were determined based on TTR (Mester 1999). Overall, non-dietary ingestion exposures were considered insignificant compare to dermal exposure (page 83) from reentry to treated turf. OEHHA agrees with the approach to calculate the exposure dosages for children with normal behavior, but suggest that ingestion exposure of children with pica should also be considered.

***Swimmer Exposure* (pages 84 to 85)**

No monitoring data for swimmers exposed to carbaryl-contaminated water was available. The dermal and oral exposures were calculated by multiplying the concentration in surface water by the dermal and oral uptake rates in children (6 years old) and adults. The carbaryl water concentrations for short-term and long-term exposures were the highest post-application levels measured in a pond and the median concentration in surface water samples, respectively. Exposure times were assumed to be 5 hours per day for short-term exposure estimates, and shorter (2.3 hours per day for children and 1.3 hours per day for adults) for long-term exposures. Default values and equations from US EPA (US EPA 2003) were used to address swimmer exposure in pools. The relevance of this approach to an outdoor swimmer scenario is difficult to assess. No information was available on frequency and duration of outdoor swimming. The EAD suggested that the concentration in surface water likely overestimated the exposure for multiple reasons (page 94). Children's exposure was considered the worse-case scenario because children have greater surface area to body weight ratio than adults (page 94). Aggregate exposure was estimated for swimmers by summing the dermal and oral exposures, while inhalation exposure was considered negligible in the outdoor setting (pages 83-85). OEHHA concurs with the assumptions and

calculations for swimmers' dermal and oral exposures to carbaryl. In California, the assumption that the weather is suitable for outdoor swimming for 100 days per year is reasonable.

Airborne Exposures Associated with Applications (pages 85 to 87)

Bystander exposures to airborne carbaryl from agricultural applications, as well as urban and suburban applications (public pest control) were calculated. The exposure rates were based on studies (Barry 2006, Wofford and Ando 2003) with a surrogate compound, methyl parathion. The use of surrogate data was justified by the observation that drift is less affected by the chemical structure of the active ingredient (AI) itself than by the application method and various physical factors (page 95). The EAD estimated exposure by multiplying the concentration in air by the uptake (page 85, Table 35) using default average breathing rates of 0.59 cubic meters per kilogram body weight per day (m^3/kg-day) for children and 0.28 m^3/kg-day for adults to calculate human exposure levels (in terms of mg/kg-day) from air concentrations.

OEHHA concurs with the use of the methyl parathion study but is concerned with the breathing rates used to calculate exposure. OEHHA recommends that DPR update its policy and consider citing the breathing rates developed for the *Air Toxics Hot Spots Program Risk Assessment Guidelines: Technical Support Document (TSD) for Exposure Assessment and Stochastic Analysis* (OEHHA 2012). In the TSD, the mean and 95th percentile daily breathing rates for infants are 0.66 and 1.09 m^3/kg-day, respectively; for adults the corresponding values are 0.19 and 0.29 m^3/kg-day.

Ambient Inhalation Exposure (page 88)

The EAD considered bystander exposure estimates to be appropriate to address ambient inhalation exposure. Exposure to ambient air was anticipated to be equal or less than the estimated bystander exposure. OEHHA concurs with this position. The comment about breathing rates for bystander exposures is also applicable to this scenario.

EDITORIAL COMMENTS

The readability of the document would be improved if the headings (sections and sub-sections) were numbered.

The equations for the calculation of absorbed daily doses were presented in different formats in the EAD. They were in footnotes under the Occupational Handler Exposure

section (for example, page 52, Table 22, footnote e). But they were in the text for the Occupational Post-Application Exposure section (for example, page 67, 4th paragraph). For clarity and consistency, OEHHA suggests a single format for all equations.

Page 35, 3rd paragraph, 4th line: Some words are missing in the sentence, "Following each application, the plots were irrigated; the with 0.3 to …"

Page 35, last paragraph, first word: "IKrolski" should be "Krolski"

Page 44, third paragraph: "A report from the California Department of Fish and Game compared carbaryl concentrations measured during surface water monitoring to concentrations found to be toxic to aquatic organisms in laboratory studies, and determined that carbaryl concentrations in the Sacramento-San Joaquin River system can present acute and chronic hazards to aquatic life (Siepmann and Jones, 1998). An assessment by DPR staff concurred (Starner, 2007)". OEHHA suggests deleting this paragraph since it is not related to the exposure assessment.

Page 46-88 EXPOSURE ASSESSMENT: This section needs better organization with additional headings, especially since it is over 40 pages long.

- An overview, similar to that presented under the heading of Occupational Post-Application Exposure (page 67), would be helpful for other subsections.

- The second and third paragraphs on page 46 discuss the exposure calculation and assumptions for workers. There was no similar information about other exposed populations such as residents and swimmers. Also this discussion seemed to be out place as it was presented before the discussion of the carbaryl concentration data that was used for dose calculation.

- The discussion of granular applications (on page 49) should be presented before Table 20, where the data are provided.

- The heading "Aerial Applications" and subsequent headings (page 50 and on) should have their own subsections, instead of immediately following the discussion of Exposure Monitoring Studies and PHED (pages 46 to 50).

Table 26: The exposures from the use of low-pressure handwand, trigger spray, and hose-end sprayer were based on a study conducted by Merricks (1997). This was noted in Table 27, and should also be noted in Table 26.

Page 67: In the equation, "ADD" should be "STADD."

Page 68: In the oxadiazon study, the body weight used for residential for post-application exposure (Table 33) was 69.4 kg, whereas 70 kg was used in other parts of the EAD (Tables 30, 31) based on this same study. Mathematically this difference is trivial but perhaps the same values should be used for consistency.

Page 84: Under "Soil Ingestion," the units for bulk soil density should be g/cm^3, not cm^3/g, and the body weight for 6 year old child (15 kg) should be specified as an assumption in the text.

Page 86 (Table 35) and page 87 (Table 36): This table cited an OEHHA 2000 reference for the hourly breathing rate. DPR should update the value, if needed, and cite the latest version of OEHHA's guidelines (OEHHA 2012).

Pages 89-90: The text (pages 89-90) which relates to Table 37 (PHED data) does not give the chemical specific exposure rates for all scenarios described. In order to compare PHED data with chemical specific data, it would be helpful to have the rates from both sources in the text or the Table, even though the references are included.

Pages 96-118: Many of the web links associated with certain references are not working. For example, the links starting with http://www.cdpr.ca.gov/docs/empm should start with http://www.cdpr.ca.gov/docs/emon. Other links, such as http://www.cdpr.ca.gov/docs/sw/contracts/usgs024100.pdf and http://www.arb.ca.gov/research/abstracts/a6-177-33.htm did not work either.

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DATE: June 10, 2014

SUBJECT: RESPONSES TO OEHHA COMMENTS ON CARBARYL EXPOSURE ASSESSMENT DOCUMENT - HS 1788

The draft exposure assessment document (EAD), for carbaryl, Health and Safety Report (HS) HS1788 was prepared by the Worker Health and Safety (WHS) Branch of the Department of Pesticide Regulation (DPR). The EAD and the associated draft risk characterization document (RCD) were sent out for external review. OEHHA provided comments on the information in the EAD. This memo contains responses to OEHHA's comments. Comments were also generated by OEHHA for the RCD. These were addressed by the Medical Toxicology Branch. DPR would like to thank OEHHA for their valuable input.

Comment 1: The abstract summarizes the key conclusions regarding risk and is not actually an abstract. Listed scenarios were identified as "having the highest level of concern" based on comparison of exposure to toxicity profile (as stated on page 88 in the RISK APPRAISAL section). OEHHA recommends that if the section continues to be named as an "abstract" it should be revised to reflect the key points from each of the sections in the EAD (environmental concentrations, pharmacokinetics, scenarios, exposure assessment…), not solely conclusions regarding risk.

The section-by-section summary suggested by this comment is more commonly found in an executive summary than in an abstract. DPR believes that the basis for the exposure assessment document and the key results are all that should be presented in the abstract. DPR keeps the abstract short to emphasize the key conclusions of the document; this is what is most useful in supporting the Department's regulatory decision process.

Comment 2: The introduction contains only two sentences on carbaryl's use and a statement about its mode of action. OEHHA recommends this section be revised to provide the reader with information on the layout and content in the EAD.

DPR agrees with this suggestion. Future EAD's will incorporate this information into the introduction section. However, rather than delay mitigation further, this and other suggested changes that do not affect its conclusions will not be made in this document.

Comment 3: The EAD describes a wide range of occupational and non-occupational scenarios including application exposure of handlers, and post-application exposure of workers, bystanders, residents (including children at play on treated lawns), and swimmers. For the exposure of workers entering a treated area (reentry) after a certain time (i.e., the reentry interval or REI), DPR used a tiered approach to select work activities. Since carbaryl is used on many crops, some of the crops were grouped and portrayed by a representative crop. For each representative crop, work activities with the highest potential for exposure were selected as scenarios for exposure calculations. OEHHA concurs with the choice of scenarios in the EAD. However, OEHHA recommends that the EAD includes exposure to carbaryl in take-home dust. There is evidence in recent studies showing that non-volatile pesticides can be found in significant amounts in homes of agricultural workers (Bradman et al. 2007, Curwin et al. 2007, Golla et al. 2012, Gunier et al. 2011) or homes neighboring agricultural fields (Gunier et al. 2011). Gunier et al. (2011) investigated the association between proximity to fields and pesticides residues in carpet dust. Significant associations were determined for simazine, phosmet, chlorpyrifos, iprodione and chlothal-dimethyl. While the study did not find a significant association for carbaryl, there were limitations to the study, including small sample size and lack of information about pesticide use patterns. In addition, the EAD should note the possibility that there are potentially multiple sources of carbaryl contributing to the levels detected in indoor dust and that an assessment of aggregate exposure may be warranted.

Consistent with U.S. EPA residential exposure assessment policy, DPR did not estimate exposure for residents potentially exposed to carpet dust contaminated with carbaryl. Exposure levels to carbaryl via this route are anticipated to be insignificant. However, the amount of dust internalized by the residential bystander could be estimated via the data of the studies conducted by Curwin et al. 2007, Golla et al. 2012, and Gunier et al. 2011. The Curwin et al. investigation generated an estimated atrazine dose via urinary samples collected from children under 16 years of age who lived on farms. The samples were collected during the spring and summer. The median weight of the children assayed in the study is 27.2 kg. The geometric mean of the data for atrazine in the urine of farm children is 13 ng/kg/day. Multiplying the body weight and the dosage provides an estimated absorbed atrazine amount of 353.6 ng/day. The Golla et al. study measured the amount of atrazine in dust samples collected from carpets, rugs, and floors in homes on farms. These samples were collected during the "growing season" (April – June). The average amount of atrazine per dust sample is 422 ng/g. Dividing the absorbed atrazine amount (i.e., 353.6 ng/day), with the amount of atrazine per dust sample (i.e., 422 ng/g), provides the estimated amount of carpet dust internalized by the child per day (i.e., 0.84 grams of dust/day). In the Gunier et al. study, the highest concentration of carbaryl measured in carpet dust from houses located within 1250 meters of agricultural crops is 113 ng/g. Multiplying this concentration with the estimated amount of dust internalized provides the daily absorbed amount

of carbaryl (i.e., 94.9 ng/day). If this value is divided by the previously mentioned median body weight of 27.2 kg, the daily dose of carbaryl is 3.5 ng/kg/day or 0.0000035 mg/kg/day. This result suggests that exposure via the take-home dust pathway is insignificant.

Comment 4: DPR followed the acceptability criteria set by US EPA (1996) to select the studies for estimating a worker's dermal exposure to DFR. The DFR studies conducted with carbaryl included dissipation studies on field crops, vegetables and fruits, and spot sampling studies. In the absence of carbaryl-specific DFRs for certain crops, DFRs from other chemicals or other crops were used as surrogates. Interpolation of DFR data for the days that were not sampled post-application was done by log-linear regression of data for sampled days; the rationale on the use of this regression was provided on page 91. The transfer coefficients (TC) were considered not chemical-specific but rather crop- and activity-specific. When data were not available, DPR used default TC values from similar cases (that is, a combination of similar activity and a similar crop). OEHHA concurs with DPR's methodology and DFR and TC values, as summarized in Table 16 (page 36). However, the studies and values may need to be updated using the current version of the US EPA guidelines (2012).

DPR is reviewing current US EPA reentry exposures guidelines and expect to adopt them in part or fully in the next year. The revised guidelines from US EPA include new data for some scenarios, and continue to use existing data for others. Any uncertainty in estimated reentry exposures from use of older data will be considered during the mitigation phase.

Comment 5: Transferable Turf Residues (TTRs) were determined to estimate the dermal exposure to carbaryl on treated turf and sod (pages 35 to 37). Carbaryl residues were measured in two studies, one using a liquid formulation and one using a granular formulation. On page 35, the introductory sentence to this section stated "Available data do not appear to support a consistent relationship between TTR and exposure." It isn't clear whether this is a general statement that applies to all pesticides or a statement that applies specifically to the TTR data that are available for carbaryl. In the summaries of the two studies, there was no explanation why these results were rejected and surrogate exposure monitoring data were used instead (page 37, first paragraph). OEHHA recommends additional explanation for rejection of the TTR studies. Furthermore, this section of the EAD should indicate why surrogate exposure data were used.

The statement in the EAD, "Available data do not appear to support a consistent relationship between TTR and exposure," applies to all pesticides tested. In the absence of information to the contrary the statement is extended to pesticides that have not yet been tested, including carbaryl. Although multiple studies support a correspondence between dislodgeable foliar residues on crops and fieldworker exposures (e.g., Zweig et al., 1985; Bruce et al., 2006), available data do not appear to support a consistent relationship between TTR and exposure (Baugher et al., 2004). This suggests that the model which works well for fieldworker exposures might not apply

to post-application exposures on turf. Hence, surrogate exposure monitoring data were used to estimate post-application exposures on turf.

Comment 6: Data from two air monitoring studies for carbaryl associated with agricultural applications were available, but were determined not acceptable because of limited sampling and lack of information about application and monitoring conditions (page 37). Instead, data from a methyl parathion airblast application (Barry 2006) was used as a surrogate to determine the air concentration for bystander exposure (pages 37 to 40). The justification was that airborne concentrations and drift depend on the equipment, timing, and location of the application, and the vapor pressure of the active ingredient rather than the chemical structure of the active ingredient (page 38). OEHHA considers the justification reasonable. However, the potential impact of a 10-fold higher vapor pressure for methyl parathion (page 38, 2nd paragraph) compared to carbaryl on the inhalation exposure estimates for bystanders should be discussed in the EAD.

The increased vapor pressure for methyl parathion would likely result in increased air concentrations. Hence, the surrogate would be health protective since the air concentrations used to estimate exposure would likely be greater than those of carbaryl, leading to higher estimated exposure.

Comment 7: Multiple studies were available that monitored on-site air in California (Table 18, page 41). The highest concentration detected in any of these studies was 12 $\mu g/m^3$ (Neher et al. 1982). Two studies were available that monitored off-site air in California (Table 19), one monitoring drift from an aerial application and one monitoring off-site air following a mist blower application. The conclusion of the study summaries provided no indication which one was selected as a basis for estimating bystander exposure from applications in urban areas. This information was provided much later on page 87 (Table 87). OEHHA suggests that the EAD include a discussion on the merits of the Neher et al. (1982) study and provide justification why it was used as a basis for calculating these exposure estimates.

The Neher et al. study was used for estimating exposure because it had the highest air concentration detected and would provide the most health-protective estimate. Moreover, the study is more realistic since it is based upon actual data.

Comment 8: No chemical-specific monitoring data were available for granular applications using a push-type lawn spreader (page 49). DPR used a "well-conducted" surrogate study by Klonne and Honeycutt (1999) with applicators using Dacthal® granular herbicide, containing 0.9 percent dimethyl tetrachloroterephthalate (the active ingredient). Dermal and inhalation exposures were estimated for push type-spreader loaders and applicators (pages 65-66, Tables 20, 28 and 29). OEHHA concurs with the use of a surrogate study to estimate exposure when no chemical specific data was available, but justification for the selection of the study conducted by Klonne and Honeycutt (1999) is needed.

The study was used as a surrogate study because it was well-conducted and had the correct formulation (i.e., granular).

Comment 9: For post-application exposure estimates, DPR determined crops where carbaryl is used, as reported in DPR's Pesticide Use Report, selected work activities that represent typical fieldworker activities for a crop group, and identified the activities with highest potential exposures (page 20, Tables 7 and 8). The studies used to calculate the DFR and TC were discussed on pages 31 to 37. OEHHA suggests that the identification of representative activities included consideration of the extent of carbaryl use. High exposure could occur during high use season. For example, pruning was identified as the representative activity for the use of carbaryl on olive trees (page 22), but according to PUR data (page 75) this activity does not occur during the months of high carbaryl use (July-August). This could have underestimated the exposure.

The representative activity of pruning was selected because, for short-term exposure, the activity was likely to generate exposure equal to or greater than that generated by the other activities it represents. For commercially grown olives, pruning is the only manual activity. The fruit is collected via mechanized harvester which shakes it from the tree. Hence, even though pruning does not occur during seasonal application of carbaryl, it has the greatest potential for exposure.

Comment 10: For workers on turf or sod after application of carbaryl, dermal exposure was assumed to occur the same day as the application because the product label did not specify a post-harvest interval (PHI) for applications to golf courses, lawns, and other turf (page 78). Results from a study of adults doing exercise on oxadiazon-treated carpet (Rosenheck and Sanchez 1995) were used as surrogate data for carbaryl to determine the exposure rate (micrograms per kilogram per hour, µg/kg-hour). Dermal exposure was adjusted using a 90 percent protection factor for covered body regions provided by long sleeves, long pants and shoes. In this scenario, the EAD only determined short term exposure because carbaryl application on turf was infrequently reported (DPR 2012). OEHHA concurs with these assumptions although the choice of oxadiazon as a surrogate needs to be justified. This study was briefly described, using the same text, on pages 79 and 82, but no details were provided on how the exposure rate was determined.

The exposure rate in µg/hour was estimated by summing oxadiazon residues from all dermal exposure matrices for each individual and dividing the result by that individual's monitoring time. Statistics including mean and 95th percentile were calculated for total dermal exposure rate.

Comment 11: Such explanation should be included as part of the presentation of studies under Transferable Turf Residues (TTR) on pages 35 to 36.

The explanation was omitted because the TTR data from the study were not used.

Comment 12: For residential handlers, a surrogate study with Dacthal® (Klonne and Honeycutt 1999) for push-type spreader application and PHED data for backpack sprayer application were used to determine the exposure rates. Data from carbaryl studies (Merricks 1997) were used for workers using handwand, trigger sprayer, hose-end sprayer, and dust can application. The exposure rate determinations assumed that all handlers (push type spreader included) wore protective clothing and chemical-resistant gloves (Table 32). OEHHA is concerned that this assumption may not be valid based on the following information. On page 17, the EAD stated "In contrast [to liquid formulations], most labels on granular products and baits have user safety recommendations rather than requirements for residential users. As users can legally choose not to follow the recommendations, exposure estimates for residential handlers of these products do not assume that protective clothing or PPE are used." Page 92 of the RISK APPRAISAL section included the following statement: "Users of pesticides are legally required to follow use directions given on pesticide labels but non-occupational pesticide handlers are not inspected for safety. In recognition of this enforcement gap, exposure estimates were calculated for users not complying with product label requirements for PPE." Furthermore, there was a large difference when exposure estimates were calculated for users NOT complying with product label requirements for Personal Protective Equipment (PPE) (Table 38). When results were compared, exposures without PPE were estimated to be 3 to 80 times higher than those with PPE (page 93). Despite the acknowledgement of a low level of enforcement, DPR still assumed PPE use in the exposure estimate calculations. OEHHA suggests the EAD present only the exposure estimates that were calculated assuming no PPE, as shown in Table 38 (page 92), with handlers wearing loose-fitting shorts and no gloves. US EPA considered that residential applicators would wear short pants, T-shirts and shoes (US EPA 1992, 2012) as a "worse case but common scenario". Carbaryl liquid applications were restricted to spot treatments of 1000 square feet and 2-4 applications/year at least 7 days apart. The maximum rate allowed was a function of the application type. Carbaryl granular/bait applications were restricted to treatments of 0.5 acre at 8.28 pounds of active ingredient per acre (lb AI/acre) with no minimum reapplication interval or applications/year. Seasonal, annual and lifetime uses were not anticipated. Only the short-term absorbed daily dose (STADD) was calculated (page 80, Table 32). OEHHA concurs with the assumptions and the calculation only for short-term exposure.

While it's possible that some residential handlers might not follow all label instructions as required by law, DPR assumes that they do. It is appropriate for DPR to estimate exposures for handlers following all label requirements, as this practice allows DPR to determine potential health concerns that could be associated with legal uses of the AI.

Comment 13: The representative reentry scenario in residential settings is dermal exposure from contact with treated lawns. Dermal exposure rates for toddlers (3-year olds) and adults on treated lawns were calculated using data from a surrogate study with adults

exposed to oxadiazon (Rosenheck and Sanchez, 1995). Adults and toddlers were expected to spend 2 hours per day on treated turf (US EPA 1997).OEHHA concurs with the assumptions that were incorporated into these calculations as shown on Table 33 (page 82), but suggests that DPR clarifies the assumptions regarding the clothing worn by children and adults. Previous comments about the oxadiazon study also apply to this section.

Residents in post-application scenarios are assumed to wear shorts. This assumption is consistent with the approach used by USEPA.

Comment 14: On page 78, DPR stated that reentry was assumed to occur on the same day as the application without any reentry interval (REI). But the study used to estimate exposure (Beauvais 2012) followed the label requirement "until the spray has dried". Depending on the weather conditions at the time of application, the drying time could last several hours, particularly along coastal areas of California. If the EAD used data from a study with REI without adjusting the result to account for a "no REI" assumption, it most likely underestimated exposure. Moreover, OEHHA notes that the assumption "until the spray has dried" is subjective and vague, adding uncertainties to the assessment. US EPA (2012) considered post-application exposure for residential users without re-entry interval. OEHHA suggests that DPR follow US EPA guidance and adjust the data so they are consistent with the EAD assumptions.

The study assumed no REI, as reentry exposure monitoring occurred on the application day after sprays dried. What this means in terms of the exposure calculation is that we use dislodgeable foliar residue (DFR) estimated for the day of application. As DFR is estimated on a daily rather than hourly basis, this means that both DPR and USEPA use DFR from the application day, and both have the same no-REI assumption.

Comment 15: In addition, the EAD should provide the data and calculations from the Beauvais (2012) study to show how the exposure rates were determined, instead of simply citing this internal report.

The detailed calculations are contained in a 23-page memo that is available on DPR's website (Beauvais, 2012).

Comment 16: Incidental non-dietary ingestion by toddlers of pesticides applied on turf was included to account for hand-to-mouth transfer, object-to-mouth transfer and soil Ingestion (pages 83-84). A carbaryl-specific non-dietary ingestion exposure monitoring study was not available. Consequently, exposure estimates were determined based on TTR (Mester 1999). Overall, non-dietary ingestion exposures were considered insignificant compare to dermal exposure (page 83) from reentry to treated turf. OEHHA agrees with the approach to calculate the exposure dosages for children with normal behavior, but suggest that ingestion exposure of children with pica should also be considered.

Consistent with USEPA policy, DPR did not calculate exposures for children ingesting an abnormally large amount of soil (i.e., pica). Exposures might be slightly underestimated for such children; however, the dermal exposure route still dominates.

Comment 17: Bystander exposures to airborne carbaryl from agricultural applications, as well as urban and suburban applications (public pest control) were calculated. The exposure rates were based on studies (Barry 2006, Wofford and Ando 2003) with a surrogate compound, methyl parathion. The use of surrogate data was justified by the observation that drift is less affected by the chemical structure of the active ingredient (AI) itself than by the application method and various physical factors (page 95). The EAD estimated exposure by multiplying the concentration in air by the uptake (page 85, Table 35) using default average breathing rates of 0.59 cubic meters per kilogram body weight per day (m^3/kg-day) for children and 0.28 m^3/kg-day for adults to calculate human exposure levels (in terms of mg/kg-day) from air concentrations. OEHHA concurs with the use of the methyl parathion study but is concerned with the breathing rates used to calculate exposure. OEHHA recommends that DPR update its policy and consider citing the breathing rates developed for the *Air Toxics Hot Spots Program Risk Assessment Guidelines: Technical Support Document (TSD) for Exposure Assessment and Stochastic Analysis* (OEHHA 2012). In the TSD, the mean and 95th percentile daily breathing rates for infants are 0.66 and 1.09 m^3/kg-day, respectively; for adults the corresponding values are 0.19 and 0.29 m^3/kg-day.

DPR's draft revised inhalation rates policy is currently in external peer review. Uncertainties and differences caused by relying on the older policy will be considered during the mitigation phase.

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cc: Ann Hanger, Environmental Scientist, Registration Branch

Bayer CropScience

October 23, 2012

Ms. Ann Hanger
California Department of Pesticide Regulation
Pesticide Registration Branch
1001 I Street
Sacramento, California 95812

Reference: Bayer's Response to CDPR'S Proposed Exposure and Risk Assessment for Carbaryl

Dear Ms. Hanger,

EXECUTIVE SUMMARY

Bayer CropScience has reviewed the draft turf and residential exposure and risk characterizations prepared by the California Department of Pesticide Regulation for the active ingredient carbaryl. As a result of its review, Bayer has identified an error that requires correction and is proposing that newer and more robust operator exposure and post application exposure data be utilized in the exposure assessment.

Based on the use of the best available exposure data, the margins of exposure used in the risk characterization of carbaryl are revised as follows:

Scenario	Draft MOE	Refined MOE
Groundboom M/L	6	61
Groundboom Applicator	102	52
High Pressure Handwand	0.24	38
LCO Push Spreader	49	49
Residential Backpack	85	85
Residential Pump Sprayer	1,615	1,615
Residential RTU Trigger Sprayer	13,394	13,394
Residential Hose End Sprayer	1,940	1,940
Residential Duster	491	50
Residential Push Spreader	50	491
Golf Course Maintenance	5	180
Adult Lawn Reentry	5	583
Toddler Lawn Reentry	3	350

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Based on the nature of the risk assessment and the conservative assumptions used, it is Bayer's conclusion that all of the refined margins of exposure should be characterized as acceptable because:

1. The exposure is based on the 95th percentile of exposure at the maximum label application rate.
2. The dermal no observed effect level of 20 mg/kg/day can be further refined using benchmark dose analysis, as conducted by the US Environmental protection Agency. The dermal $BMDL_{10}$ calculated by EPA was 30.56 mg/kg/day.

I. INTRODUCTION

The California Department of Pesticide Regulation (DPR) issued a draft occupational and bystander risk characterization document for carbaryl on 12 July 2012. A human exposure assessment document[1] dated 28 June 2012 was prepared and provides the human exposure assessments that form the basis for the risk characterization document. HS-1788 provides a comprehensive assessment of the potential exposure from occupational and residential uses of carbaryl and also to incidental exposures resulting from agricultural drift to residential premises or from swimming in waters containing carbaryl from agricultural uses. The DPR assessment evaluated the short-term exposure potential resulting from single use of carbaryl, seasonal exposure, and long-term exposure including the carcinogenic potential of carbaryl.

Bayer CropScience currently maintains or supports registrations for residential uses of carbaryl and for professional applications to golf course turf. Bayer has divested the agricultural uses of carbaryl. Therefore, the purpose of this assessment is to provide DPR with corrections and refinements for the human exposure assessment to the residential and golf course uses of carbaryl. This assessment does not address the agricultural uses or carbaryl or the incidental exposure potential to bystanders or swimmers resulting from the agricultural uses.

II. SUMMARY OF DPR EXPOSURE AND RISK CHARACTERIZATION

The human health exposure assessment was prepared by the Worker Health and Safety Branch for inclusion in the risk characterization document for carbaryl because of adverse effects resulting from acute inhibition of acetylcholinesterase activity and tumorigenesis reported in laboratory studies. Thus, DPR concluded that the exposures that are potentially of most concern included short-term exposures and lifetime exposures approaching the level implicated in tumorigenesis.

The handler scenarios relevant to turf and residential uses include mixing/loading and groundboom application of liquid formulations to golf course turf, mixing/loading and hand-held sprayer application of liquid formulations to golf course turf, loader/applicator

[1] Beauvais, S. Human Exposure Assessment Document for Carbaryl. HS-1788. 28 June 2012.

use of the granular formulation by push spreaders to golf course turf or residential lawns by a lawn care operator (LCO), homeowner application of the granular formulation to lawns with a push spreader, and homeowner application of liquids or dusts to residential ornamentals and gardens by hose-end sprayers, hand pump sprayers, backpack sprayers, ready-to-use (RTU) trigger sprayers, or dust shaker cans. The relevant post application exposure scenarios are golf course maintenance workers and residents re-entering either lawns treated with the granular formulation or reentry to treated ornamental or vegetable garden areas.

For the groundboom application of carbaryl DPR focused on crop uses and assumed 80 acres/day are treated for non-high acre crops at an application rate of 2.0 lb a.i./A. The short-term absorbed daily dose (STADD) for the groundboom mixer/loader was 2.33 mg/kg/day based on DPR's use of the 95th percentile of the unit exposure, a 70% dermal absorption, and a 70 kg body weight. The STADD for the groundboom applicator was 0.146 mg/kg/day.

A hand-spray application of carbaryl to golf course turf was based on the high-pressure hand-held sprayer scenario from PHED. The application of 1000 gallons per day was assumed with spray concentration of 0.156 lb a.i./gallon. The STADD for the high pressure hand-spray mixer/loader/applicator was 42.6 mg/kg/day.

The exposure of an LCO applying the granular formulation of carbaryl to residential turf was based on a carbaryl specific study in which a push spreader was used. The STADD was estimated as 0.261 mg/kg/day based on the application rate of 8.28 lb a.i./A to 5 acres/day.

With the exception of backpack sprayers the residential exposure assessments were based on carbaryl-specific exposure studies previously conducted by Rhone-Poulenc. These studies monitored the exposure during the use of carbaryl products by hose-end sprayers, low-pressure pump sprayers, dust applicators, RTU trigger sprayers, and push spreaders. In addition the exposure from the use of backpack sprayers was assessed using data from PHED. The STADD for the residential home uses of carbaryl were as follows.

- Backpack 0.163 mg/kg/day
- Low Pressure Pump Sprayer 0.00794 mg/kg/day
- RTU Trigger Sprayers 0.000946 mg/kg/day
- Hose-End Sprayer 0.00708 mg/kg/day
- Duster 0.0261 mg/kg/day
- Push Spreader 0.146 mg/kg/day

DPR assessed the post application exposure of carbaryl to turf for both golf course maintenance workers and residential lawn reentry based on the exposure data of volunteers monitored doing a Jazzercise routine following an oxadiazon application to turf at the application rate of 3.0 lb a.i./A. DPR did not rely on any carbaryl-specific turf transferable residue (TTR) data because DPR does not believe that a consistent relationship exists between measured TTRs and turf post application exposure.

The dermal exposures for occupational scenarios, assumed that reentry workers wore long-sleeved shirt, long pants, and shoes and were calculated by assuming a 90% protection factor for covered body regions. The mean exposure rate, adjusted to the maximum application rate of 8.28 lbs AI/acre for carbaryl (i.e., after multiplying by 8.28/3.0), is 345 µg/kg/hour. The 95th percentile exposure rate is 489 µg/kg/hour. The STADD was calculated from the 95th percentile exposure rate assuming 70% dermal absorption and an 8-hour workday. The STADD was estimated to be 2.74 mg/kg/day for golf maintenance workers.

For residential post application lawn exposure DPR did not adjust the dosimeter residues for clothing. The mean dermal exposure rate for adults was 1,390 µg/kg/hour and the 95th percentile exposure rate was 1,840 µg/kg/hour. The STADD was calculated to be 2.58 mg/kg/day assuming 2 hours of exposure and a 70% dermal absorption. The dermal exposure for a 15 kg toddler was also estimated from the oxadiazon study by adjusting the exposure rate for the difference in body weights and surface area between the study participants and a standard 15 kg child. The 95th percentile exposure rate for a 15 kg toddler was calculated as 3,090 µg/kg/hr. The STADD based on 2 hours of exposure and a 70% dermal absorption value was 4.33 mg/kg/day for the toddler. DPR considered incidental non-dietary oral exposure from toddler contact with the treated lawn. Based on their calculations the contribution from the oral route was insignificant compared to the dermal exposure and the total STADD remained at 4.33 mg/kg/day.

Based on the exposure assessment presented in HS-1788, the risk characterization document calculated the margins of exposure (MOEs) for carbaryl. A dermal NOEL of 14 mg/kg/day was selected for the short-term exposure assessment based on the carbaryl 28-day rat dermal toxicity study NOEL of 20 mg/kg/day and adjusting for a 70% dermal absorption to estimate the systemic equivalent dose. It should be noted that benchmark dosing can refine the dermal NOEL. Based on the EPA assessment the $BMDL_{10}$ for dermal toxicity was calculated as 30.56 mg/kg/day. Bayer believes that the $BMDL_{10}$ is a more refined characterization of the dermal toxicity point of departure and is superior to the study-based dose level NOEL of 20 mg/kg/day. The NOEL used for inhalation exposure was 1.0 mg/kg/day. Based on these toxicity endpoints and the STADDs calculated in HS-1788 the MOEs relevant for Bayer CropScience's carbaryl uses are as follows from the risk characterization document.

Scenario	MOE
Groundboom M/L	6
Groundboom Applicator	102
High Pressure Handwand	0.24
LCO Push Spreader	49
Residential Backpack	85
Residential Pump Sprayer	1,615
Residential RTU Trigger Sprayer	13,394
Residential Hose End Sprayer	1,940
Residential Duster	491

Residential Push Spreader	50
Golf Course Maintenance	5
Adult Lawn Reentry	5
Toddler Lawn Reentry	3

III. REFINEMENT OF GROUNDBOOM FOR GOLF COURSE USE

The exposure and risk to individuals applying carbaryl to golf course turf was not directly addressed. Carbaryl can be applied to golf course fairways by small groundboom equipment. The DPR assessment was geared toward agricultural crops such as vegetables and assumed an application to 80 acres/day. The area treated to specifically address golf course applications should be refined to 40 acres/day. The maximum application rates for carbaryl to turfgrass is either 4 lb a.i./acre or 8 lb a.i./acre, depending on the pest type.

DPR relied upon the PHED open pour liquid formulation mixer/loader scenario (Scenario 5) to obtain the unit exposure dermal and inhalation exposure estimates of 1,446 µg/lb a.i. and 7.34 µg/lb a.i. for the 95th percentile of exposure. PHED was not designed to provide reliable upper percentile estimates of exposure and the scenario has been formally replaced by EPA with data developed by the Agricultural Handler Exposure Task Force (AHETF). DPR has been a partner with EPA, PMRA, and the AHETF in the development of handler exposure to replace PHED and Bayer CropScience as a member of the AHETF believes that the open pour mixer/loader AHETF data should be used by DPR as the most reliable data available. On 11 March 2011, EPA issued its revised monograph of the open pour liquid formulation mixer/loader data (DP Barcode D373605). Table 1 of the EPA monograph is reproduced below and provides the exposure estimates calculated by EPA for use in assessing open pour mixer/loader exposure.

Table 1. Unit Exposures (ug/lb ai handled): Open Pour Mixing and Loading Liquid Formulations

Exposure Route	PHED	AHETF[a, b]		
	"Best Fit"	Geometric Mean	Arithmetic Mean[c]	95th Percentile[d]
Dermal	23	19.8	37.6	127.5
Inhalation	1.2	0.083	0.219	0.822

[a] Dermal unit exposures reflect 50% adjustment of hand and face/neck measurements. The average percent of dermal exposure representing the hands, face, and neck is 46%.
[b] Statistics are estimated using a variance component model accounting for correlation between measurements conducted within the same field study (i.e., measurements collected during the same time and at the same location). Additional model estimates (e.g., empirical and simple random sample assumptions) are described in Section III.
[c] Arithmetic Mean (AM) = GM * exp{0.5*((lnGSD)^2)}
[d] 95th percentile = GM * GSD^1.645

Based on the EPA estimates of exposure, Bayer believes that DPR should refine its open pour mixer/loader assessment for golf course use based on the dermal and inhalation unit

exposure estimates of 127.5 µg/lb a.i. for dermal exposure and 0.822 µg/lb a.i. for inhalation exposure. The use of the 95th percentile would be consistent with DPR policy.

The golf course mixer/loader STADD is calculated as follows for the application rate of 4 lb a.i./A. A 70 kg body weight, 70% dermal absorption, and treatment of 40 acres/day is assumed.

Dermal Exp: 0.1275 mg/lb a.i. x 40 A/day x 4 lb a.i./A x 0.7 ÷ 70 kg = 0.204 mg/kg/day
Inhal. Exp.: 0.000822 mg/lb a.i. x 40 A/day x 4 lb a.i./A ÷ 70 kg = 0.0019 mg/kg/day

The resultant open pour mixer/loader MOEs are as follows.
Dermal MOE: 14 mg/kg/day ÷ 0.204 mg/kg/day = 69
Inhalation MOE: 1 mg/kg/day ÷ 0.0019 mg/kg/day = 526
Combined MOE: 61

While the combined MOE does not exceed 100, the refinement in the application rates, acres treated, and exposure data provide a more reliable risk estimate for DPR risk management decision making.

The applicator exposures can be similarly refined by replacing the DPR PHED based unit exposures of 85.3 µg/lb a.i. for dermal exposure and 4.12 µg/lb a.i. for inhalation exposure with the AHETF open-cab groundboom unit exposures. On 11 March 2011, EPA issued its revised monograph of the open cab groundboom applicator data (DP Barcode D373605). Table 1 of the EPA monograph is reproduced below and provides the exposure estimates calculated by EPA for use in assessing open cab groundboom exposure.

Table 1. Unit Exposures (ug/lb ai handled): Open Cab Ground Boom Application of Liquid Sprays

Exposure Route	PHED	AHETF[a, b]		
	"Best Fit"	Geometric Mean	Arithmetic Mean[c]	95th Percentile[d]
Dermal	14	6.9	16.1	58.5
Inhalation	0.74	0.12	0.34	1.27

[a] Dermal unit exposures reflect default 50% adjustment of hand and face/neck measurements to account for potential exposure method collection inefficiencies. The average percent of dermal exposure representing the hands, face, and neck is 37.6%.
[b] Statistics are estimated using a variance component model accounting for correlation between measurements conducted within the same field study (i.e., measurements collected during the same time and at the same location). Additional model estimates (e.g., empirical and simple random sample assumptions) are described in Section III.
[c] Arithmetic Mean (AM) = GM * exp{0.5*((lnGSD)^2)}
[d] 95th percentile = GM * GSD^1.645

Based on the EPA estimates of exposure, Bayer believes that DPR should refine its open cab groundboom applicator assessment for golf course use based on the dermal and inhalation unit exposure estimates of 58.5 µg/lb a.i. for dermal exposure and 1.27 µg/lb a.i. for inhalation exposure. The use of the 95th percentile would be consistent with DPR policy.

The golf course groundboom applicator STADD is calculated as follows for the maximum application rate of 8 lb a.i./A. A 70 kg body weight, 70% dermal absorption, and treatment of 40 acres/day is assumed.

Dermal Exp: 0.059 mg/lb a.i. x 40 A/day x 8 lb a.i./A x 0.7 ÷ 70 kg = 0.19 mg/kg/day
Inhal. Exp.: 0.00127 mg/lb a.i. x 40 A/day x 8 lb a.i./A ÷ 70 kg = 0.0058 mg/kg/day

The resultant open-cab groundboom applicator MOEs are as follows.
Dermal MOE: 14 mg/kg/day ÷ 0.19 mg/kg/day = 74
Inhalation MOE: 1 mg/kg/day ÷ 0.0058 mg/kg/day = 172
Combined MOE: 52

While the MOE does not exceed the 100 value typically used with assessments conducted with mean exposures, Bayer believes that the assessment is a more accurate assessment that is specific to the golf course use for use in risk management decision-making.

IV. REFINEMENT OF HANDWAND APPLICATIONS FOR GOLF COURSE USE

The exposure and risk to individuals applying carbaryl to golf course turf by hand held sprayers was also not directly addressed. Carbaryl can be applied to golf course greens by hand held sprayers attached by a hose to the spray tank. DPR relied upon the PHED high pressure hand held sprayer M/L/A scenario (Scenario 21) to obtain the unit exposure dermal and inhalation exposure estimates of 26,470 µg/lb a.i. and 565 µg/lb a.i. for the 95th percentile of exposure. The scenario has been formally replaced by EPA with data developed by the Outdoor Residential Exposure Task Force (ORETF). DPR has been a partner with EPA, PMRA, and the ORETF in the development of handler exposure to replace PHED and Bayer CropScience as a member of the ORETF believes that the turf hand held sprayer mixer/loader/applicator data should be used by DPR as the most reliable data available. The ORETF study is OMA002, Exposure of Professional Lawn Care Workers During the Mixing and Loading of Dry and Liquid Formulations and the Liquid Application of Turf Pesticides Utilizing a Surrogate Compound dated 22 January 1999. Fifteen individuals were monitored mixing/loading and applying a liquid formulation to turf. Each volunteer operated a TruGreen-ChemLawn truck during the study. The trucks were either closed box trucks called "Rhinos", or larger, more open designed trucks called "Hippos". The "Hippo" and "Rhino" trucks are TruGreen-ChemLawn equipment and are typical of equipment used in the lawn care industry. Sprayers on the trucks were operated at approximately 125 psi on 400 ft of hose with an electric rewind reel. All of the sprayers were equipped with twin line, 2 stop nozzle guns (Lesco® 2 gallon nozzle). This equipment is representative of the hand gun sprayer applications that are made to golf course turf. The dermal and inhalation exposure data for the 15 replicates were obtained from Tables 2A and 3A of the study report and used to calculate the 95th percentile population exposure estimate for individuals wearing long-sleeved shirts, long pants, and protective gloves.

The geometric mean dermal and inhalation exposures normalized for both the individual's body weight and amount of active ingredient handled are 5.32 and 0.018 µg/kg bw/lb a.i., respectively. The geometric standard deviations are 3.3 and 2.32, respectively. The maximum measured dermal and inhalation exposures among the 15 replicates were 28.9 µg/kg bw/lb a.i. for dermal exposure and 0.053 µg/kg bw/lb a.i. for inhalation exposure. The 95th percentile of population exposure was calculated based on the formula that the 95th percentile = GM*GSD^1.645. For the assessment of golf course handwand applications the unit exposures at the 95th percentile of 37.9 µg/kg bw/lb a.i. for dermal exposure and 0.074 µg/kg bw/lb a.i. for inhalation exposure are used.

In the ARTF Study 057 golf course maintenance worker reentry study the greens averaged 0.09 acres each. Based on 18 greens the total acreage is estimated to be about 1.6 acres. Because the handwand applications are primarily to greens a total of 1.6 acres is assumed to be treated at a maximum application rate of 8.28 lb a.i./acre. A 70% dermal absorption is also assumed. The STADD is calculated as follows.

Dermal Exp: 0.038 mg/kg bw/lb a.i. x 1.6 A/day x 8.28 lb a.i./A x 0.7 = 0.35 mg/kg/day
Inhal. Exp.: 0.000074 mg/kg bw/lb a.i. x 1.6 A/day x 8.28 lb a.i./A = 0.001 mg/kg/day

The resultant MOEs are as follows.

Dermal MOE:	14 mg/kg/day ÷ 0.35 mg/kg/day = 40
Inhalation MOE:	1 mg/kg/day ÷ 0.001 mg/kg/day = 1,000
Combined MOE:	38

While the MOE does not exceed the 100 value typically used with assessments conducted with mean exposures, Bayer believes that the assessment is a more accurate assessment that is specific to the golf course handwand use for use in risk management decision-making.

V. PUSH SPREADER USE

DPR calculated the exposure to LCOs and homeowners applying the granular formulation of carbaryl by push-spreaders. The data are based on the ORETF study OMA004. Bayer concurs that this is the best available data and its use is consistent with Bayer's position that ORETF study OMA002 is the best available data for the handwand LCO application. The STADD for LCO use of the push spreader is based on treating 5 acres/day and for homeowner use it is based on treating 0.5 acres/day. Bayer notes that Table 32 of the draft DPR exposure assessment has transposed the STADD values for the homeowner dust application and the push-spreader applications. The total STADD exposure for the push spreader is 0.0261 mg/kg/day but it is presented as the dust value. Consequently, the STADD presented for dust applications should be the 0.146 mg/kg/day that is currently presented as the push-type spreader STADD.

The MOE of 49 for the LCO push spreader use presented in Table IV-7a of the risk characterization document has been reproduced by Bayer. However, the homeowner

push-spreader and homeowner dust MOEs presented in Table IV-8a of the risk characterization document are also transposed based on the transposition that occurred in Table 32. The MOE for homeowner push-spreader use should be 491 and for homeowner dust use the MOE should be 50.

VI. TURF MAINTENANCE POST APPLICATION EXPOSURE

The turf maintenance assessment for golf course workers were estimated using data from a surrogate study in which exposure was monitored during choreographed activities on turf following an application of oxadiazon (Rosenheck and Sanchez, 1995). The oxadiazon study monitored exposure of 10 volunteers performing a 16-minute Jazzercise® routine on turf treated with a liquid oxadiazon product at a rate of 3.0 lbs AI/acre. Dermal exposure was monitored with outer whole-body dosimeters, cotton gloves, hand washes, and face/neck wipes. DPR assumed that for the dermal exposures for occupational scenarios that reentry workers wear long-sleeved shirt, long pants, and shoes; exposures were calculated by assuming a 90% protection factor for covered body regions. The mean exposure rate calculated by DPR, adjusted to the maximum application rate of 8.28 lbs AI/acre for carbaryl (i.e., after multiplying by 8.28/3.0), was 345 µg/kg/hour. The 95th percentile exposure rate was 489 µg/kg/hour.

Bayer does not believe that the Jazzercise® routine is in any way representative of the activities conducted by golf course maintenance workers. It is Bayer's position that the golf course maintenance worker exposure study conducted by the Agricultural Reentry Task Force (ARTF)[2] is the appropriate study to base risk management decisions for carbaryl post application exposure for golf course workers. The study was reviewed and accepted by EPA on 6 March 2006 (DP Barcode 327361) and the analysis provided in this Bayer assessment is derived from the EPA assessment.

The purpose of this study was to estimate potential dermal and inhalation exposures and calculate dermal transfer coefficients among golf course workers re-entering a treated golf course to perform various golf course maintenance tasks. The tasks monitored included cup changing, greens mowing, greens watering, fairway mowing, irrigation repair, and miscellaneous grooming. Two trials were conducted at an 18-hole golf course near Hood River. The first trial was conducted on the first 9 holes and the second trial was conducted on the back nine holes. In both trials, each greenway or fairway section was treated once with Daconil Weather Stik® Flowable Fungicide Turf Care® Turf and Ornamental Fungicide containing the active ingredient (ai) chlorothalonil. The soluble concentrate product was applied using commercial ground boom sprayers at a rate of approximately 5.5 lb ai/A. The application for the second trial took place two days after the application for the first trial. Exposures were monitored for each activity on one and two days after application. The potential dermal exposures were assessed by using whole-body dosimetry (inner and outer), hand washes, and face/neck wipes. The potential

[2] Determination of Dermal and Inhalation Exposure to Reentry Workers During Maintenance Activities in Golf Courses. Study ARF057. 5 August 2005.

inhalation exposures were assessed by using personal air sampling pumps attached to OVS tubes. A total of three to four replicates per re-entry interval (trials 1 and 2 combined) were collected. For each replicate, the work period was approximately 1.5 to 4.5 hours long.

To remain consistent with the DPR methodology Bayer has calculated the dermal exposures for each worker monitored by summing the dermal exposures for each body area presented in Tables 6, 7, and 8 of the EPA assessment report. The total dermal exposure in µg for each worker was normalized by the duration of the worker's monitoring duration to provide the exposure as µg/hr. Because a worker can be expected to conduct any one of the activities following a carbaryl application and because there did not appear to be a difference in the exposures occurring on Day 1 compared to Day 2, the data were combined for all work activities and both days of monitoring. The exposures are presented as follows.

Day 1 Data from EPA DER Tables 6 - 8

Replicate	time (hr)	Inner Dos (µg)	F/N (µg)	Hand (µg)	TDE (µg)	TDE (µg/hr)
CC1	1.6	1900	0.5	419	2319.5	1450
CC2	2.2	365	0.5	125	490.5	223
CC4	1.82	17.3	1.54	389	407.84	224
GM1	1.63	88.6	0.5	297	386.1	237
GM2	1.52	58.7	0.5	230	289.2	190
GM5	2.7	441	3.17	324	768.17	285
GM6	2.57	3945	1.06	425	4371.06	1701
GW1	3.02	354	0.5	660	1014.5	336
GW2	2.83	292	0.5	649	941.5	333
GW5	2.32	256	1.78	558	815.78	352
GW6	2.28	29.4	0.5	308	337.9	148
FM1	2.22	177	1.45	367	545.45	246
FM2	4	3837	1.55	167	4005.55	1001
FM5	4.47	2142	5.47	2238	4385.47	981
FM6	2.6	145	1.55	1189	1335.55	514
IR1	2.68	1454	0.5	482	1936.5	723
IR2	3.4	424	0.5	17.5	442	130
IR5	3.62	4649	1.27	424	5074.27	1402
IR6	3.08	310	0.5	384	694.5	225
MG1	3.48	11019	0.5	193	11212.5	3222
MG4	3.22	117	0.5	209	326.5	101
MG5	2.77	213	1.9	672	886.9	320
					mean	652

Day 2 Data from EPA DER Tables 6 - 8

Replicate	time (hr)	Inner Dos (µg)	F/N (µg)	Hand (µg)	TDE (µg)	TDE (µg/hr)
CC3	2.68	160	0.5	143	303.5	113
CC5	1.67	23	0.5	205	228.5	137
CC6	1.27	171	0.5	171	342.5	270
GM3	2.97	91	0.5	29.7	121.2	41
GM4	2.22	25.5	1.25	224	250.75	113
GM7	2.87	140	0.5	150	290.5	101
GM8	2.2	16.2	0.5	83.5	100.2	46
GW3	3.12	199	1.18	49.2	249.38	80
GW4	3	78	2.42	135	215.42	72
GW7	2.23	109	0.5	383	492.5	221
GW8	2.1	22.7	0.5	168	191.2	91
FM3	3.6	738	3.26	42.3	783.56	218
FM4	2.02	61.2	2.37	295	358.57	178
FM7	3.97	175	1.54	427	603.54	152
FM8	2.27	30.4	0.5	327	357.9	158
IR3	2.98	860	2.41	42.3	904.71	304
IR4	3.03	1044	2.08	274	1320.08	436
IR7	3.65	163	0.5	147	310.5	85
IR8	3.22	4877	0.5	145	5022.5	1560
MG2	2.72	693	0.5	87.6	781.1	287
MG3	3.32	315	3.65	171	489.65	147
MG6	3.12	205	3.33	241	449.33	144
					mean	225

The geometric mean dermal exposure of the 44 monitoring units is 251 µg/hr with a geometric mean standard deviation of 2.69. The 95th percentile of the population is calculated using the same equation that EPA uses to calculate the 95th percentile for the AHETF scenarios (95th percentile = GM*GSD^1.645). Based on this equation the 95th percentile of exposure is 1,280 µg/hr. The 95th percentile of exposure is based on the study application rate of 5.5 lb a.i./acre and requires adjustment for the carbaryl maximum turf application rate of 8.28 lb a.i./acre. The adjustment factor is 1.5. Therefore, the 95th percentile of exposure for golf course maintenance workers following a carbaryl application is 1,920 µg/hr.

Golf course maintenance workers typically work on the greens, tees, and fairways for not more than 4 hours/day before doing other activities. Therefore, the STADD is based on a 4 hour/day exposure duration, a 70% dermal absorption, and a 70 kg bodyweight and is calculated as follows. The MOE is based on the NOAEL of 14 mg/kg/day.

STADD: 1,920 µg/hr x 4 hrs/day x 0.7 ÷ 70 kg = 76.8 µg/kg/day = 0.077 mg/kg/day
MOE: 14 mg/kg/day ÷ 0.077 mg/kg/day = 180

It is Bayer's position that the assessment of golf course maintenance worker risk should be based on the ARTF golf course maintenance worker reentry study and not a Jazzercise based reentry study. This refinement to risk assessment demonstrates that worker exposure after application is acceptable.

VII. LAWN TURF POST APPLICATION EXPOSURE

The oxadiazon turf reentry study was also used by DPR to estimate the post application exposure to residential turf following a lawn broadcast application. The Jazzercise® routine used in the oxadiazon study is considered appropriate for estimating the post application exposure of toddlers and adults following home lawn applications. However, the formulation used in the oxadiazon study was a liquid formulation and only the granular formulation of carbaryl is permitted for residential turf broadcast applications.

The ORETF has conducted a post application turf exposure study[3] following a granular formulation application that Bayer believes is more appropriate for conducting an exposure and risk assessment for a granular residential turf application. DPR was involved in the development of the study protocol and the final report has previously been submitted to DPR by ORETF.

ORF 030 was conducted with adult participants to measure the potential for dermal deposition of residues during reentry to a turf area treated with a pesticide. Two separate types of activities were included in this study: an approximate 20-minute Jazzercise® routine and a 2-hour composite routine composed of many typical children's activities. This composite routine of children's' activities was called the CHAPS routine (Children's Activity Patterns).

The turf was treated with a granular or a liquid formulation of the surrogate active ingredient, dithiopyr, formulated as the Dimension® emulsifiable concentrate or the Dimension® 270-G granule. The test site was a field at the Grant County fairgrounds in Moses Lake, Washington. The application for the liquid-treated plot was made with a commercial groundboom sprayer; the granule-treated plot was made with a metered-feed drop spreader on dry grass. The application rate was approximately 0.5 lb ai/acre for both the liquid and granule applications. The liquid application was made with an application volume of 30 gal/A; the granule application was made at 165 lb granular product per acre.

The CHAPS routine was a series of 12 sequential activities that simulated activities in which children routinely engage on residential turf. Both the activities and duration of

[3] Determination of Potential Dermal Exposure to Adults and Children Reentering a Pesticide-Treated Turf Area. Study ORF030. 23 September 2003.

this routine were considered to constitute an upper bound estimate of exposure potential. The following table summarizes the activities and the time allotted for those activities.

Activity	Duration (minutes)
Walking/Jogging	12
Playing catch	12
Crawling	12
Picnicking	12
Playing with toys	8
Playing Frisbee	8
Playing soccer	8
Playing games (spud)	8
Playing tag (steal the bacon)	8
Resting	12
Football	10
Tumbling	10

The potential dermal exposures for both the liquid and granular formulations normalized for the duration of exposure were obtained from Table 8 of the ORF030 study report and are presented below.

Summary of Residue Levels Normalized for Time of Routine for Various Clothing Scenarios for the Jazzercise® and CHAPS Routines on Turf Treated with Granular and Liquid Formulations

RESIDUE LEVEL (µg/hr)[a]

CLOTHING SCENARIO	Jazzercise - Granular	Jazzercise – Liquid	CHAPS – Granular	CHAPS – Liquid
		SESSION 1		
LS-LP	10.8	143	13.5	169
TS-SH	22.2	849	34.1	1070
PDE	46.2	2770	51.0	2350
CLOTHING SCENARIO	**Jazzercise - Granular**	**Jazzercise – Liquid**	**CHAPS – Granular**	**CHAPS – Liquid**
		SESSION 2		
LS-LP	10.2	48.9	15.0	87.5
TS-SH	19.2	240	35.0	425
PDE	36.0	753	51.0	870

The Jazzercise® and CHAPS routines gave similar estimates of exposure within each of the two formulation types. The results also show that the exposure potential following the application of a liquid formulation is completely different than the exposure potential following a granular application. This supports Bayer's position that the granular data from ORF030 is more appropriate than the oxadiazon liquid formulation data used by DPR.

The individual monitoring data for potential dermal exposure that were provided in Appendix 4, Table 3A or study report ORF030 were used to calculate the 95th percentile of the population exposure using the equation GM*GSD^1.645. The toddler exposure was calculated by adjusting the adult exposure with the same adjustment factors used by DPR with the oxadiazon study. Therefore, a 69.4 kg adult body weight and 15 kg toddler body weight were assumed as was an adult body surface are of 18,150 cm^2 and a toddler surface area of 6,565 cm^2 to yield an adjustment factor of 1.67. The estimated toddler exposure is 1.67 times greater than the measured adult exposure from the study. Based on the data from the ORF030 study the geometric mean adult exposure was 0.66 µg/kg/hr with a GSD of 1.32. The geometric mean toddler exposure was 1.09 µg/kg/hr with a GSD of 1.32._The 95th percentiles of exposure for the adult and toddlers were 1.04 µg/kg/hr and 1.74 µg/kg/hr, respectively.

An adjustment of 16.6 for the difference between the dithiopyr application rate of 0.5 lb a.i./acre used in study ORF030 and the maximum carbaryl application rate of 8.28 lb a.i./acre is used to estimate the 95th percentile of exposure for carbaryl granular applications to residential turf. The resulting exposure estimates are 17.3 µg/kg/hr for adults and 28.9 µg/kg/hr for toddlers. These exposure estimates are used along with the DPR assumptions of a 2-hour duration of exposure and a 70% dermal absorption to calculate the adult and toddler STADDs. The MOEs are calculated based on the NOAEL of 14 mg/kg/day.

Adult Post Application Residential Turf Exposure and Risk
STADD: 17.3 µg/kg/hr x 2 hr/day x 0.7 = 24 µg/kg/day = 0.024 mg/kg/day
MOE: 14 mg/kg/day ÷ 0.024 mg/kg/day = 583

Toddler Post Application Residential Turf Exposure and Risk
STADD: 28.9 µg/kg/hr x 2 hr/day x 0.7 = 40 µg/kg/day = 0.040 mg/kg/day
MOE: 14 mg/kg/day ÷ 0.040 mg/kg/day = 350

Based on refinement of the turf post application exposure assessment by utilizing granular formulation exposure data in place of liquid formulation data the MOEs for adults and toddlers on the turf following a carbaryl granular application exceeds 100.

VIII. CONCLUSION

Bayer CropScience has reviewed the draft carbaryl exposure and risk assessments prepared by DPR as part of the re-evaluation process in California. This Bayer review focused solely on the non-dietary exposure assessment and does not address in any detail

the hazard characterization prepared by DPR. In addition, the Bayer review does not address the DPR policy regarding acute toxicity endpoint risk assessments and the use of the 95th percentile. Based on the review of the DPR assessments Bayer believes that the following issues require consideration by DPR.

1. Refine the occupational exposure assessments based on PHED scenarios with the data developed by the Agricultural Handlers Exposure Task Force. DPR has been involved in the development of these data and they are the best data available. Therefore the AHETF data should be used to assess the exposure during open-pour mixing/loading and during open-cab groundboom applications. In additions Bayer has refined the application rate from 2 lb a.i./acre for field crops to 8.28 lb a.i./acre for turf and has refined the area treated to be specific for turf.
2. Refine the hand-held spray applications that are currently based on PHED with data developed by the Outdoor Residential Exposure Task Force. DPR has been involved in the development of the ORETF data and they are superior to the PHED data. Therefore, the ORETF hand gun application data should be used to assess the hand-held applications of carbaryl to golf course turf. The application rate has also been refined to be specific to turf.
3. Correct the transposition of the STADD and MOEs for push-spreader applications with the dust applications for both professional turf and residential turf applications of carbaryl.
4. The use of the oxadiazon turf re-entry exposure study is not appropriate for assessing the post application exposure of golf course maintenance workers. The assessment should be based on a study that the Agricultural Reentry Exposure Task Force has conducted and submitted to DPR monitoring the exposure to workers conducting golf course maintenance activities. A study monitoring the actual work activities is more appropriate than an assessment based on the Jazzercise® routine.
5. The use of the oxadiazon turf re-entry exposure study is based on the application of a liquid formulation to turf. Only the granular formulation of carbaryl is registered for residential lawn application and a turf residential post application exposure study conducted by the Outdoor Residential Exposure Task Force with a granular formulation is more appropriate. Therefore, the ORETF granular post application exposure data should be used to assess residential lawn post-application exposure.
6. Benchmark dose analysis can be used to refine the dermal endpoint from 20 mg/kg/day to a $BMDL_{10}$ of 30.56 mg/kg/day.

Based on these refinements to the draft DPR risk assessments the MOEs for carbaryl golf course and residential uses are refined as follows.

Scenario	Draft MOE	Refined MOE
Groundboom M/L	6	61
Groundboom Applicator	102	52
High Pressure Handwand	0.24	38
LCO Push Spreader	49	49

Residential Backpack	85	No change
Residential Pump Sprayer	1,615	No change
Residential RTU Trigger Sprayer	13,394	No change
Residential Hose End Sprayer	1,940	No change
Residential Duster	491	50
Residential Push Spreader	50	491
Golf Course Maintenance	5	180
Adult Lawn Reentry	5	583
Toddler Lawn Reentry	3	350

Bayer believes that the above refinements to the exposure assessment are consistent with DPR policy and provides a more accurate evaluation for risk management decisions regarding the potential exposure and risk while maintaining consistency with DPR policy. All recalculations were performed based on the dermal no observed effect level of 20 mg/kg/day (adjusted using 70% dermal absorption), but using the $BMDL_{10}$ of 30.56 mg/kg/day for the dermal endpoint would further refine the assessment.

Acknowledgement
Curt Lunchick with Bayer CropScience LP contributed the contents of this document. Thank you.

Please contact me directly regarding the subject matter, Office 919-549-2435, Email julio.rosa@bayer.com.

Thank you,

Julio Rosa, Jr.
State Registration Specialist

Department of Pesticide Regulation

Brian R. Leahy
Director

Edmund G. Brown Jr.
Governor

MEMORANDUM

TO: Tom Moore, Ph.D.
Acting Branch Chief
Medical Toxicology Branch
Dept. of Pesticide Regulation, Cal-EPA

FROM: Andrew Rubin, Ph.D., D.A.B.T.
Staff Toxicologist, Health Assessment Group
Medical Toxicology Branch
Dept. of Pesticide Regulation, Cal-EPA

DATE: June 20, 2014

SUBJECT: RESPONSE TO BAYER CROPSCIENCE COMMENTS ON THE DRAFT CARBARYL OCCUPATIONAL / BYSTANDER RISK CHARACTERIZATION DOCUMENT

In a memo dated October 23, 2012, Bayer CropScience offered only one recommendation concerning DPR's draft carbaryl occupational / bystander risk characterization document (dated July 12, 2012). That recommendation and DPR's response appear below.

Note: Many other comments in Bayer's memo concerned the draft Exposure Assessment Document (dated June 28, 2012). DPR responses to those comments appear in a separate memo.

Bayer comment---page 15: "6. Benchmark dose analysis can be used to refine the dermal endpoint from 20 mg/kg/day to a $BMDL_{10}$ of 30.56 mg/kg/day."

DPR response: DPR opted to retain the critical dermal endpoint NOEL of 20 mg/kg/day, which was based on a 50 mg/kg/day LOEL and adjusted to 14 mg/kg/day due to 70% dermal absorption, over a benchmark dose derived value. The NOEL dose was, after all, experimentally tested, and not one inferred through extrapolation. Moreover, the proximity between the NOEL and LOEL doses testified to the accuracy of the NOEL. In conclusion, the NOEL/LOEL approach was considered superior to the benchmark dose approach in this particular case.

Department of Pesticide Regulation

Brian R. Leahy
Director

Edmund G. Brown Jr.
Governor

MEMORANDUM

TO: Lisa Ross, Ph.D., Chief
Environmental Program Manager II
Worker Health and Safety Branch

VIA: Sheryl Beauvais, Ph.D. *(original signed by S. Beauvais)*
Senior Toxicologist
Worker Health and Safety Branch

FROM: Ian Reeve, Ph.D. *(original signed by I. Reeve)*
Staff Toxicologist (Specialist)
(916) 323-7617

DATE: January 28, 2014

SUBJECT: RESPONSES TO COMMENTS FROM BAYER CROPSCIENCE ON CARBARYL EXPOSURE ASSESSMENT DOCUMENT - HS1788

The draft exposure assessment document (EAD), Health and Safety Report (HS) HS1788, for carbaryl was prepared by the Worker Health and Safety (WHS) Branch of the Department of Pesticide Regulation (DPR). The EAD and the associated draft risk characterization document (RCD) were sent out for external review. Bayer provided comments on the information in the EAD. This memo contains responses to Bayer's comments. Comments were also generated by Bayer for the RCD. These were addressed by the Medical Toxicology Branch. DPR would like to thank Bayer for their valuable input.

Comment 1: Refine the occupational exposure assessments based on PHED scenarios with the data developed by the Agricultural Handlers Exposure Task Force. DPR has been involved in the development of these data and they are the best data available. Therefore the AHETF data should be used to assess the exposure during open-pour mixing/loading and during open-cab groundboom applications. In additions [sic] Bayer has refined the application rate from 2 lb a.i./acre for field crops to 8.28 lb a.i./acre for turf and has refined the area treated to be specific for turf.

DPR is reviewing the databases mentioned and working to revise its defaults. The additional uncertainties will be considered during the mitigation phase.

Comment 2: In additions [sic] Bayer has refined the application rate from 2 lb a.i./acre for field crops to 8.28 lb a.i./acre for turf and has refined the area treated to be specific for turf.

DPR estimates exposure under highest-exposure conditions for each scenario. If these exposures do not indicate a potential health concern, then those involving lower application rates and acres treated will also be considered as not indicating a potential health concern. Conversely, if the

exposure suggests a potential health concern, then factors such as application rate, size, and uncertainties in exposure and risk will be considered during the risk management phase.

Comment 3: Refine the hand-held spray applications that are currently based on PHED with data developed by the Outdoor Residential Exposure Task Force. DPR has been involved in the development of the ORETF data and they are superior to the PHED data. Therefore, the ORETF hand gun application data should be used to assess the hand-held applications of carbaryl to golf course turf. The application rate has also been refined to be specific to turf.

DPR is working to update our handler exposure estimates to include newer data submitted by the ORETF, and factors including use directions on individual product labels and uncertainties in exposure and risk estimates will be addressed in mitigation.

Comment 4: Correct the transposition of the STADD and MOEs for push-spreader applications with the dust applications for both professional turf and residential turf applications of carbaryl.

The transposition error for the STADD values mentioned in comment number 3 was addressed and the corrections made in the EAD.

Comment 5: The use of the oxadiazon turf re-entry exposure study is not appropriate for assessing the post application exposure of golf course maintenance workers. The assessment should be based on a study that the Agricultural Reentry Exposure Task Force has conducted and submitted to DPR monitoring the exposure to workers conducting golf course maintenance activities. A study monitoring the actual work activities is more appropriate than an assessment based on the Jazzercise® routine.

The difference in activities monitored in the two studies mentioned increases uncertainty in the exposures estimated for golf maintenance exposures. However, as explained in the exposure appraisal section of the EAD, "The ARTF conducted a study of golf course maintenance workers intended to address exposures during typical golf course maintenance activities (Klonne and Bruce, 2005); however, exposures were highly variable and may have been affected by changing levels of moisture on the grass, making the study results difficult to interpret."

Comment 6: The use of the oxadiazon turf re-entry exposure study is based on the application of a liquid formulation to turf. Only the granular formulation of carbaryl is registered for residential lawn application and a turf residential post application exposure study conducted by the Outdoor Residential Exposure Task Force with a granular formulation is more appropriate. Therefore, the ORETF granular post application exposure data should be used to assess residential lawn post-application exposure.

Some liquid formulations of carbaryl registered in California still carry use instructions on product labels allowing applications to residential turf, as well as use in recreational areas such as parks, where post-application exposure to children might be anticipated. For this reason, exposures were assessed assuming liquid applications.

Comment 7: Benchmark dose analysis can be used to refine the dermal endpoint from 20 mg/kg/day to a BMDL10 of 30.56 mg/kg/day.

Comment number 7 does not apply to the EAD.

cc: Ann Hanger, Environmental Scientist, Registration Branch