Australian Pesticides &
Veterinary Medicines Authority

The reconsideration of registrations of products containing carbaryl and their approved associated labels

Part 1:

Uses of carbaryl in home garden, home veterinary, poultry and domestic situations

FINAL REVIEW REPORT AND REGULATORY DECISION

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ABBREVIATIONS

Time

d	Day
h	Hour
min	Minute
mo	Month
wk	Week
s	Second
yr	Year

Weight

bw	Body weight
g	Gram
kg	Kilogram
µg	Microgram
mg	Milligram
ng	Nanogram
wt	Weight

Length

cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre
nm	Nanometre

Dosing

id	Intradermal
im	Intramuscular
inh	Inhalation
ip	Intraperitoneal
iv	Intravenous
po	Oral
sc	Subcutaneous
mg/kg bw/d	mg/kg bodyweight/day

Volume

L	Litre
mL	Millilitre
µL	Microlitre

Concentration

M	Molar
ppb	Parts per billion
ppm	Parts per million

Clinical chemistry, haematology

A/G	Albumin/globulin ratio
ALT	Alanine aminotransferase (SGPT)
AP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
BUN	Blood urea nitrogen
ChE	Cholinesterase
CPK	Creatine phosphatase (phosphokinase)
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
LH	Luteinising hormone
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NTE	Neurotoxic target esterase
PCV	Packed cell volume (Haematocrit)
PT	Prothrombin time
RBC	Red blood cell/erythrocyte
T_3	Triiodothyroxine
T_4	Thyroxine

TSH	Thyroid stimulating hormone (thyrotropin)
WBC	White blood cell/leucocyte
WBC-DC	White blood cells – differential count

Anatomy

CNS	Central nervous system
GIT	Gastro-intestinal tract

Chemistry

DMSO	Dimethyl sulfoxide
GC	Gas chromatography
GLC	Gas liquid chromatography
HPLC	High pressure liquid chromatography
MS	Mass spectrometry
RIA	Radioimmunoassay
TLC	Thin layer chromatography

Terminology

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
GD	Gestation Day
GLP	Good Laboratory Practice
LD	Lactation Day
LOEL	Lowest Observed Effect Level
MRL	Maximum Residue Limit or Level
NOEL	No Observed Effect Level
NOAEL	No Observed Adverse Effect Level
OP	Organophosphorus pesticide

Organisations & publications

ACPH	Advisory Committee on Pesticides and Health
APVMA	Australian Pesticides and Veterinary Medicines Authority
CAC	Codex Alimentarius Commission
ECETOC	European Chemical Industry Ecology and Toxicology Centre
FAO	Food and Agriculture Organisation of the UN
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
JECFA	FAO/WHO Joint Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
NCI	National Cancer Institute
NDPSC	National Drugs and Poisons Scheduling Committee
NHMRC	National Health and Medical Research Council
NTP	National Toxicology Program
OCS	Office of Chemical Safety (within the TGA Group of Regulators)
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

1. TOXICOLOGY ASSESSMENT: REVIEW OF THE MAMMALIAN TOXICOLOGY AND METABOLISM/TOXICOKINETICS OF CARBARYL

1.1 TOXICOLOGY HAZARD PROFILE

1.1.1 Absorption, distribution, metabolism and excretion in mammals

Rate and extent of absorption	Oral absorption is rapid and extensive in humans, rodents and other species. Dermal absorption from aqueous media is slow and saturable in rodents but enhanced in the presence of organic solvents. Pulmonary absorption is rapid.
Distribution	Small amounts in carcass, kidney and liver.
Potential for accumulation	Very low.
Rate and extent of excretion	Rapid, extensive, predominantly via urine in all species except dog.
Metabolism	Rapid. Proceeds via hydrolysis, alkyl oxidation, arene oxide formation, epoxide hydrolysis and glutathione conjugation. Pathways similar in humans, rodents and other species investigated.
Toxicologically significant metabolites	Reactive epoxide intermediates may be formed in mice and rats.

1.1.2 Acute toxicity

Rat oral LD_{50} (mg/kg bw)	246
Worst oral LD_{50} in other species	150 mg/kg bw in cats
Rat dermal LD_{50} (mg/kg bw)	No data
Worst dermal LD_{50} in other species	>2000 mg/kg bw in rabbits
Rat inhalation LC_{50} (mg/m^3)	2500 (4h) as aerosol
Worst inhalation LC_{50} in other species	No data
Eye irritation	Classified as slight in rabbits
Skin irritation	Classified as not irritating in rabbits
Skin sensitisation	None in guinea pigs

1.1.3 Metabolites of carbaryl

Rat oral LD_{50} (mg/kg bw)	
4-hydroxycarbaryl	1190
5-hydroxycarbaryl	297
7-hydroxycarbaryl	4760
hydroxymethylcarbaryl	>5000
1-naphthol	2570

1.1.4 Short-term toxicity

Target/critical effect	ChE depression, cholinergic symptoms
Lowest relevant oral NOEL (mg/kg bw/d)	1 in rats (13-wk neurotoxicity study by gavage)
Lowest relevant dermal NOEL (mg/kg bw/d)	No data
Lowest relevant inhalation NOEL (mg/m^3)	10 in rats (90-d study, highest dose tested)

1.1.5 Genotoxicity

Clastogenic *in vitro* but not *in vivo*.
Interrupts spindle formation *in vitro*.
Overall weight of evidence lies against mutagenicity or genotoxic activity by other mechanisms.

1.1.6 Long term toxicity and carcinogenicity

Target/critical effect	Kidney: cloudy swelling of tubules
Lowest relevant NOEL (mg/kg bw/d)	1.8 in 1-yr dog study by gavage
Carcinogenicity	Vascular tumours in male mice in a 2-yr dietary study at 16 mg/kg bw/d, the lowest dose tested. At the highest dose (1350 mg/kg bw/d), there was also development of renal adenoma and carcinoma in males, while hepatic adenoma and carcinoma became elevated in females. At the high dose of 420 mg/kg bw/d in a 2-yr rat dietary study, there was treatment-related formation of urinary bladder papilloma/carcinoma in both sexes, renal carcinoma and thyroid adenoma/carcinoma in males, and hepatic adenoma in females.

1.1.7 Reproductive toxicity

Reproduction target/critical effect	Decreased parental bw gain, bw, feed consumption and conversion efficiency, depressed gestation and lactation bw in dams, and increased pup mortality (NOEL=4.7 mg/kg bw/d).
Developmental target/critical effect	Skeletal and visceral abnormalities in dogs at and above 6.3 mg/kg bw/d in the absence of maternal toxicity.
Lowest relevant developmental NOEL	Developmental toxicity: NOEL=3.1 mg/kg bw/d. Both results in dogs.

1.1.8 Delayed neurotoxicity	No effects
1.1.9 Immunotoxicity	No data
1.1.10 Dermal absorption	In rat: Up to 2% of applied dose over 30 min, rising to a maximum of 25% at 24 h. Results obtained with formulated product applied in aqueous CMC vehicle. In humans: Up to 4.4% over 4 h and16% over 8 h, applied in acetone vehicle.

1.1.11 Summary	**NOEL**	**Study**	**Safety Factor**
Revised ADI 0.008 mg/kg bw/d, based on vascular tumour formation.	16 mg/kg bw/d*	2-yr dietary study in mice	2000[#]
Acute RfD 0.01 mg/kg bw based on ChE inhibition, clinical signs, and reduced bw gain.	1 mg/kg bw/d	13-wk neurotoxicity and developmental neurotoxicity studies by gavage in rats	100

*LOEL value.

[#]The safety factor incorporates a 10-fold component for interspecies extrapolation, a 10-fold component for intraspecies variability, a 5-fold component for adequacy of the database, and a 4-fold component for seriousness of the carcinogenic response. (This 4-fold component is comprised of a 1-fold factor [low degree of confidence that carbaryl is genotoxic], a 4-factor [medium degree of confidence that carbaryl causes malignant tumours] and a further 1-fold factor [metastases not reported]).

1.2 SUMMARY

1.2.1 Background

Carbaryl is a carbamate effective against a broad range of insects, mites, lice, millipedes and other pests. Carbaryl was reviewed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1963, 1965, 1966, 1967, 1969 and 1973. The original ADI of 0-0.02 mg/kg bw/d was set in 1963 on the basis of a NOAEL of 1.8 mg/kg bw/d in a 1-yr dog study. This was revised to 0-0.01 mg/kg bw/d in 1969 because of concern about effects on the male reproductive system seen in a 1-yr gavage study in rats with a NOAEL of 2 mg/kg bw/d, and because a dose of 0.12 mg/kg bw/d may have affected renal function in a 6-wk study in humans. In 1973, the Meeting established a full ADI of 0-0.01 mg/kg bw/d.

During the early to mid 1990s, the sponsoring company undertook a number of studies intended to modernise the toxicological database on carbaryl, which was by then approximately 30 years old. Significant concerns were raised at a national and international level by findings of the oncogenic activity in mice and rats in replacement 2-yr studies performed by Hamada (1993a, 1993b) at Hazleton laboratories. These results were in contrast to the earlier carcinogenicity studies, which had proven negative.

Consideration of these data by the UK Committee on Carcinogenicity (COC) led to removal of home carbaryl-based garden and home veterinary products from the British market by the MAFF Pesticide Safety Directorate. The US EPA imposed exposure mitigation measures on carbaryl based products pending completion of a review of the compound; an updated Toxicology Chapter for Re-registration Eligibility Decision and a Human Health risk Assessment have been released recently (US EPA 2002a, b).

Evaluation of the Hamada studies, other replacement data, historical control data and mechanistic toxicology and metabolism studies has been undertaken by the OCS Chemical Product Assessment Section and Chemical Review and International Harmonisation Section from 1994 onwards. Consideration of these evaluations by the Advisory Committee on Pesticides and Health (ACPH) occurred in October 1998. ACPH agreed with OCS's view that there were treatment-related vascular tumours in male mice at the lowest dose tested, and reduced the Australian ADI for carbaryl from 0.01 to 0.004 mg/kg bw/d by applying a 4000-fold safety factor to the LOEL of 16 mg/kg bw/d. The ACPH further recommended that the Australian Pesticides and Veterinary Medicines Authority (APVMA) [formerly the National Registration Authority for Agricultural and Veterinary Chemicals] should discontinue the registration of home garden and home veterinary products containing carbaryl, based on the lack of sufficient information from which to estimate human exposure arising from the chemical's use within a domestic setting.

Internationally, the JMPR carried out a further toxicological review of carbaryl in 1996, and decreased the ADI to 0.003 mg/kg bw/d by application of a 5000-fold safety factor to the LOEL for vascular tumours in male mice. The JMPR again considered carbaryl in 2001. The ADI was revised upwards to 0.008 mg/kg bw/d; while the basis for the ADI was unchanged, the safety factor was relaxed to 2000. The JMPR also established an ARfD for carbaryl of 0.2 mg/kg bw, based on a NOAEL for ChE inhibition of 125 ppm (equal to 3.8 mg/kg bw/d) in a 5-wk dietary study in dogs. A safety factor of 25 was applied because ChE inhibition by carbaryl [in rats] is "rapidly reversible and driven by the peak concentration in plasma."

This toxicological evaluation examines (1) supplementary studies intended to elucidate the mechanism of tumour formation, (2) replacement multi-generation reproduction and developmental studies in rats and rabbits, (3) addenda to a previously evaluated developmental neurotoxicity study in rats, (4) a short-term repeat-dose study and a 1-yr study in dogs, and (5) exposure studies undertaken on persons using American registered carbaryl products in domestic settings.

OCS's CRIH Section has also estimated the systemic doses likely to be delivered to users of Australian registered carbaryl products, under Australian conditions. These estimates have been related to toxicological benchmarks to support recommendations on the continued registration and conditions of use of carbaryl home garden and veterinary products, including safety directions.

1.2.2 Kinetics and Metabolism

Valles (1999) conducted a metabolism study in male mice which received a 50 mg/kg bw gavage dose of radiolabelled carbaryl following 14 d administration of carbaryl in the diet at 0, 10, 100, 1000 or 8000 ppm, equivalent to approximately 1.5, 15, 150 and 1200 mg/kg bw/d. Pre-treatment dose levels did not influence the excretion of radioactivity, 80% of which appeared in the urine. Up to 21 radioactive components were detected in the urine, in which the major metabolites were dihydrodihydroxy naphthyl sulfate, hydroxycarbaryl glucuronide/dihydrodihydroxy carbaryl, alpha-naphthyl sulfate and alpha-naphthyl glucuronide. Pretreatment at 8000 ppm elicited increases in production of dihydrodihydroxy naphthyl sulfate and hydroxycarbaryl glucuronide/dihydrodihydroxy carbaryl, which are believed to be formed via epoxide intermediates. The 8000 ppm group excreted approximately 25% of the administered radioactivity in the form of these urinary metabolites, compared to 17% by the non-pretreated animals. At 8000 ppm there was also a decline in the urinary excretion of some unidentified metabolites, possibly formed by alkyl oxidation. Pretreatment with 10 and 100 ppm carbaryl appeared to inhibit the hydrolytic pathway of metabolism. However, levels of the major hydrolysis products alpha-naphthyl sulfate and alpha-naphthyl glucuronide in the 1000 and 8000 ppm groups' urine, were similar to values from the non-pretreated group, accounting for about 30% of administered radioactivity.

1.2.3 Short-term Repeat-dose Study

Hamada (1991) administered carbaryl technical to dogs in the diet at concentrations of 0, 20, 45 or 125 ppm for approximately 5 wk. The study included measurement of plasma and RBC activity prior to treatment and on study days 14 and 32, and brain ChE activity at termination. A probable treatment-related depression of plasma ChE activity occurred in 125 ppm males and females, which displayed up to a 23% reduction compared with baseline activity. Statistical significance against controls was achieved on d 14 but not subsequently, due mainly to a decline in ChE activity among controls. There were no treatment-related effects on RBC or brain ChE activity, or on gross necropsy findings. Consequently, the NOEL is set at 45 ppm (equal to 1.4 mg/kg bw/d).

1.2.4 Chronic Study

Hamada (1987) administered carbaryl technical to beagle dogs in the diet at concentrations of 0, 125, 400 or 1250 ppm for 12 mo. Mean achieved doses were approximately 3.5, 11 and 34

mg/kg bw/d for males and 3.8, 11 and 36 mg/kg bw/d for females at 125, 400 and 1250 ppm, respectively. Bodyweight gain was inhibited to a biologically significant extent at 1250 ppm during the first 5 wk of treatment, accompanied (in females only) by reduced feed consumption, particularly between wk 1 and 5. Leucocyte and segmented neutrophil counts became statistically and biologically significantly elevated in 1250 ppm males. Carbaryl caused dose-related inhibition of ChE activity at all 3 feeding levels in females, and at 400 and 1250 ppm in males. Plasma ChE inhibition *vs* control was 47-66% at 1250 ppm ($p<0.05$ throughout the study), was 9-36% at 400 ppm ($p<0.05$ throughout the study in males and at 5, 13 and 26 wk in females), and was 12-23% in 125 ppm females ($p<0.05$ at 5, 13 and 26 wk). RBC ChE inhibition *vs* control was 30-56% at 1250 ppm ($p<0.05$ throughout the study) and 19-34% at 400 ppm ($p<0.05$ at 5, 13 and 26 wk in females but only at 5 and 13 wk in males). Brain ChE activity was depressed by 14-32% in the treated male groups but failed to attain statistical significance against control, while treated females showed 20-36% inhibition which was dose-related and significant ($p<0.05$) at all doses. The female 1250 ppm group had slight but significant ($p<0.05$) depression in albumin concentration at all measured time points, together with increased inorganic phosphorus at wk 52. Absolute and relative liver weights were increased in 1250 ppm males. There were no treatment-related gross or histopathological findings. Based on statistically significant depression of plasma and brain ChE activity in females treated at the lowest dose of 125 ppm (approximately 3.8 mg/kg bw/d), the study is considered not to have demonstrated a NOEL.

1.2.5 Carcinogenicity Studies

A subchronic carcinogenicity study was performed by Chuzel (1999) in male "knockout" mice, heterozygous for the p53 tumour suppressor gene. The mouse strain (C57Bl/6 Tac fBR-[KO]Trp53N5-T) is phenotypically normal, but has enhanced susceptibility to genotoxic events. Carbaryl was administered via the diet at concentrations of 0, 10, 30, 100, 300, 1000 or 4000 ppm (equal to 1.8, 5.2, 17.5, 52, 165 and 717 mg/kg bw/d) for 180 d. Carbaryl did not induce mortality or clinical signs. Treatment-related observations were confined to the 4000 ppm group, which displayed a slight but significant ($p<0.01$ *vs.* control) deficit in food consumption, correlated with lower mean bodyweight ($p<0.05$ or 0.01 *vs.* control). At study termination the 4000 ppm group mean bw remained approximately 8% below the control value. A transient decrease in food consumption among the 1000 ppm group ($p<0.05$ *vs.* control) was not accompanied by decreased growth or bw. An increase was noted in absolute and relative liver and kidney weights at 1000 and 4000 ppm, while depression in thymus weight occurred at 4000 ppm only. Statistical significance ($p<0.05$ or 0.01 *vs.* control) was attained with respect to most of these parameters. Globular deposits in the upper (umbrella) cell layer of the urinary bladder epithelium affected many animals at 100 ppm or greater. The relative severity of accumulation was dose related, but there was no accompanying irritation or hyperplastic response. The NOEL was 30 ppm (equal to 5.2 mg/kg bw/d), based on the presence of deposits in the urinary bladder epithelium at and above the next highest dose of 100 ppm. There was no treatment-related tumourigenesis.

In a study validating use of p53 knockout mice for investigating vascular tumour development (Bigot, 1999), heterozygous (+/-) males were gavaged daily with urethane at 1, 10 or 100 mg/kg bw/d for 180 d. Seventeen/20 animals from the 100 mg/kg bw/d group died prematurely, mainly from internal haemorrhage. The entire 1 mg/kg bw/d group survived, while there were 2 intercurrent deaths at 10 mg/kg bw/d. Histopathology revealed hepatic angiectasis at 10 and 100 mg/kg bw/d, and vascular neoplasia in the livers of 18/20 mice receiving 100 mg/kg bw/d, together with single occurrences of hemangiosarcoma of the

spleen and abdominal cavity and cardiac hemangioma. The 10 mg/kg bw/d group showed 1 case of multiple hepatic hemangioma. Other treatment-related tumours comprised subcutaneous sarcoma and lymphoma at 10 and 100 mg/kg bw/d, and adenoma of the lung and hepatocellular carcinoma at 100 mg/kg only. No neoplasms were present in the 1 mg/kg bw/d group. A negative control group gavaged with 250 mg/kg bw/d d-limonene displayed inappetence, mononuclear cell infiltration of the renal peripelvis and slight to moderate hyperplasia of the non-glandular stomach, but no treatment-related neoplasia. Comparison between vehicle control groups of p53 heterozygous and wild type (p53 +/+) mice showed that the genetic difference between these strains did not affect spontaneous tumour formation.

In a published review, Venkatachalam *et al*. (2001) discuss the biological and molecular mechanisms underlying enhanced cancer formation in mice heterozygous for the gene coding for the p53 protein (p53+/- mice). The p53+/- mouse strain contains one wild-type allele, together with an inactive mutant gene coding for p53. Over a half of the tumours collected from these mice retain an intact wild-type allele, while in the remainder, the wild-type allele had become completely deleted. Tumours arising at less than 18 mo of age tend to have a higher frequency of complete p53 allele loss than those arising later in the mouse life span. P53 +/- tumours that retain the wild-type allele also retain sensitivity to apoptosis following irradiation, and display other markers of p53 functionality. Compared with cells from p53 +/+ animals, fibroblasts derived from p53 -/- mouse embryos show a higher growth rate, saturation density, and less cell cycle arrest response following exposure to ionising radiation. The +/- genotype has growth characteristics and radiation response intermediate between those of the +/+ and -/- genotypes. Thus, it appears that the p53 protein is "haploinsufficient": loss of a single copy of the wild-type allele is sufficient to impair (but not prevent) the protein's tumour suppression activity. This finding is unexpected, as it has hitherto been believed that loss of *both* copies of a tumour suppressor gene are a prerequisite for tumour formation. Tumours from carcinogen-treated p53+/- mice do not reveal any consistent relationship between the carcinogen's mode of action, and whether the tumours retain or lose the remaining wild-type p53 allele. The authors suggest that the target tissue itself may have some influence over the loss or retention of the wild-type p53 allele. They conclude that carcinogenesis in the p53 +/- mouse model is likely to involve numerous carcinogen-tissue interactions that determine the likely site of tumour origin, tumour formation latency, the oncogenic lesions responsible for tumour formation, the cell-signaling pathways affected, and whether or not the wild-type p53 allele becomes inactivated.

Debruyne (1998) performed cellular proliferation studies on the kidney and liver of mice previously exposed to carbaryl for 52 weeks in a dietary study (Hamada, 1993b). Cell turnover was measured in tissue from the control and 8000 ppm interim sacrifice groups, by staining for Proliferating Cell Nuclear Antigen (PCNA). The mean number of PCNA-positive renal cortical tubular cells in 8000 ppm males (3.9/1000) was approximately 3-fold higher than in control male kidney (1.2/1000). In control females, the rate of PCNA-positive hepatocytes (mean=4.6/1000) was approximately half the mean positive staining rate among the 8000 ppm group (8.3/1000). These data suggest a higher amount of cellular replication in the kidney of male mice and the liver of females receiving 8000 ppm carbaryl, compared with controls. There is an apparent correlation between this parameter and Hamada's (1993b) finding of renal and hepatic tumours in the 8000 ppm males and females, respectively. By contrast, there was no biologically significant enhancement of cell turnover in the liver of 8000 ppm males or kidney of 8000 ppm females, which were not sites of tumour development.

Irisarri (1996) measured cellular proliferation by PCNA staining in the liver, urinary bladder and thyroid gland of rats that had been exposed to carbaryl for 52 wk in a dietary study (Hamada, 1993a). There was a small increase in cell cycling activity in the male thyroid and female liver at 7500 ppm. Although of equivocal biological significance, this finding does correlate with elevated incidences of thyroidal adenoma and hepatic adenoma in 7500 ppm males and females, respectively, in the chronic dietary experiment. A 10-fold increase in cell cycling in the urinary bladder epithelium of 7500 ppm males was of clear biological significance and correlates with the hyper- and neoplastic response observed by Hamada (1993a) within this group.

In a discussion paper, Cohen (1995) agrees with the registrant's position that carbaryl causes renal and urinary bladder tumours in rodents via a non-genotoxic mechanism. He considers it likely that the bladder tumours observed in rats at 7500 ppm resulted from a direct mitogenic effect by carbaryl or its metabolites on the urinary epithelium. Cohen's argument is based on his (1994) mechanistic study with another aromatic carbamate, propoxur, which has also been shown to cause urinary bladder cancer in rats at a high (8000 ppm) dietary dose. Cohen demonstrated cellular proliferation in the absence of necrotic injury, formation of calculi, amorphous precipitates, or crystals. With regard to the proliferative lesions seen in the male rat kidney at 7500 ppm, Cohen also attributes these to mitogenic stimulus. The author concludes that without knowing the exact mechanism involved in rats, or the route of carbaryl metabolism in humans, it was impossible to predict whether cancer of the urinary tract could occur in humans. However, given that urinary tract hyperplasia/neoplasia are restricted to rats and require dietary exposure exceeding the MTD, such lesions are unlikely at the anticipated levels of human exposure. In this respect, Dr Cohen's conclusions are consistent with the position taken by the Australian reviewing toxicologist in OCS's 1998 evaluation.

1.2.6 Reproductive Studies

In a 2-generation reproduction study (Tyl *et al.*, 2001), rats were treated with carbaryl technical in their diet at concentrations of 0, 75, 300 or 1500 ppm for a 10-wk period, and throught mating, gestation and lactation of the resulting F1 litter. The procedure was repeated with the F1 pups, which were treated at the same doses until the end of lactation of the F2 litter. The NOEL for effects on the parental generations was 75 ppm, based on the following findings at and above the next highest dose: decreased bw gain, bw, feed_consumption and conversion efficiency in F0 and F1 adults of both sexes, combined with depression in gestational bw and lactational bw and feed consumption in F1 females. A single 1500 ppm F0 male was found to be producing 100% non-motile sperm that had abnormal morphology. The 1500 ppm F0 group mean sperm motility was reduced and there was a small increase in the proportion of abnormal sperm at 1500 and possibly 300 ppm. However, there were no similar findings in F1 adults. Carbaryl did not affect the sex ratio, or growth or survival of F1 or F2 foetuses *in utero*, and did not cause malformations or clinical signs among pups during lactation. However, F1 and F2 pup growth was reduced and mortality was increased during lactation at 1500 ppm. F2 pup mortality was also enhanced at 300 ppm. Puberty was significantly retarded in both sexes at 1500 ppm. The NOEL for effects on pups was therefore 75 ppm (approximately 4.7 mg/kg bw/d), based on increased mortality during lactation of the F2 litters at and above 300 ppm.

1.2.7 Developmental Studies

Repetto-Larsay (1998) administered carbaryl by gavage to mated female rats at 0, 1, 4 or 30 mg/kg bw/d on d 6 – 20 inclusive of presumed gestation. No premature mortality occurred. At 30 mg/kg bw/d, most dams had at least one occurrence of increased salivation within 20 min of dosing, and this group also showed significantly ($p<0.01$) depressed food consumption, a transient loss of bw at the commencement of dosing, an 8% deficit (*vs.* control) in terminal bw, and significant ($p<0.01$) reduction in cumulative gross and net (without uterus) bw gain. Foetal survival and sex ratio were not compromised but there was evidence of foetotoxicity at 30 mg/kg bw/d, seen as a 13% reduction in gravid uterine weight, a significant ($p<0.01$) deficit in foetal bw, an increased incidence of runts, and delayed ossification of the spinal vertebrae and paw. However, there were no treatment-related visceral anomalies or malformations. The NOEL was 4 mg/kg bw/d, based on maternotoxicity (salivation and depressed food consumption and bw gain) and foetotoxicity (reduced foetal bw and delayed ossification) at the highest dose of 30 mg/kg bw/d.

In a range finding study by Tyl (1999), carbaryl was administered by gavage to mated female rabbits at 0, 3, 7.5, 20, 50 or 100 mg/kg bw/d on d 6 – 29 inclusive of presumed gestation. No treatment-related clinical signs or unscheduled deaths were observed. There was a significant ($p<0.05$) trend towards decreasing maternal bw gain over the dosing period, with group mean values being reduced by about 20% at 50 and 100 mg/kg bw/d. A parallel trend occurred in net maternal bw change when corrected for gravid uterine weight. At 100 mg/kg bw/d, ChE activity was inhibited by 20% in RBC and 59% in plasma ($p<0.05$) relative to control values. There was no effect on foetal survival or development. Although a near significant ($p=0.0506$) trend towards dose related depression in foetal bw occurred, attributable to a 16% reduction (*vs.* control) at 100 mg/kg bw/d, the finding was of equivocal biological significance. As this is a range finding study employing limited group sizes and limited observations, a NOEL will not be set.

Tyl, Marr and Myers (1999) gavaged mated female rabbits with 0, 5, 50 or 150 mg/kg bw/d carbaryl on d 6 – 29 inclusive of presumed gestation. The 150 mg/kg bw/d group lost weight over gestation day (gd) 6 – 9, and displayed significantly ($p<0.01$) depressed cumulative bw gain over the dosing and entire gestation periods. When corrected for gravid uterine weight, maternal net bw loss was nearly 3-fold higher at 150 mg/kg bw/d than among controls. ChE activity was inhibited dose-relatedly at 50 and 150 mg/kg bw/d ($p<0.01$). At the mid and high doses, respectively, ChE inhibition amounted to approximately 46 and 68% in plasma and 19 and 29% in erythrocytes. Treatment did not compromise foetal survival or sex ratio, but caused significant ($p<0.01$) depression in foetal bodyweight at 150 mg/kg bw/d. However, there were no effects on foetal development. The NOEL for maternal effects was 5 mg/kg bw/d, based on plasma and RBC ChE inhibition at and above the next highest dose of 50 mg/kg bw/d. The NOEL for foetotoxicity was 50 mg/kg bw/d, based on depressed bodyweight at the highest dose of 150 mg/kg bw/d.

1.2.8 Neurotoxicity Studies

In amendments to a developmental neurotoxicity study by the same authors, which was evaluated by OCS in 1998, Robinson and Broxup (2001a & b) performed additional morphometric analyses on the forebrain and cerebellum of 11- and 70-d old offspring from the control dams and dams receiving 10 mg carbaryl/kg bw/d.by gavage from GD 6 to 10 d

post-partum. The additional measurements had been recommended in a US EPA assessment which indicated a possible treatment-related decrease in the length and weight of the cerubellum in 11 d old female offspring of dams treated at 10 mg/kg bw/d, together with a bilateral increase in the width of the cerebellum in 70 d old female offspring from the same group. [The OCS assessment did not ascribe toxicological significance to these findings because of contradictory sex- and time-related changes from control.] The findings of these two supplementary studies were entirely negative with respect to all measured parameters, and do not change the OCS's original conclusion that there were no neurotoxic or developmental effects on pups at the highest dose (10 mg carbaryl/kg bw/d). The maternal NOEL remains at 1 mg/kg bw/d (based on reduced bw gain, autonomic effects, tremors and ChE depression at the highest dose).

1.2.9 Human Studies

A series of user exposure studies was performed, in which untrained volunteers applied various carbaryl based home garden/veterinary insecticides while wearing a long sleeved cotton shirt, long cotton pants and a whole body dosimeter under the outer clothing. The amount of carbaryl deposited on the clothing, inner dosimeter, hands, face and neck was measured by HPLC. Breathing zone air was also sampled and assayed for the active constituent.

During application of a 5.4% powder insecticide product to 3 dogs, volunteers were exposed dermally and inhalationally to a mean of 1111 and 7986 μg carbaryl, respectively, when wearing or not wearing gloves. When adjusted for volunteer bodyweight and the amount of active constituent used, carbaryl exposure was 4.8 and 36 μg/kg bw/g applied, under the respective conditions (Merricks, 1997a).

When ungloved volunteers applied a 22.4% liquid product to vegetables, the mean exposure to carbaryl was 836 μg and 247 μg, using hose-end and hand-held pump sprayers, respectively. When adjusted for bodyweight and the amount of active constituent used, carbaryl exposure was 0.5 and 0.4 μg/kg bw/g applied, with the respective sprayer types. If gloves were worn, total exposure was reduced to 7 μg and 5.9 μg (0.004 and 0.011 μg/kg bw/g applied), with hose-end and hand held pump sprayers, respectively (Merricks, 1997b).

Application of the 22.4% liquid product to 2 large and 2 small trees, caused volunteers to be exposed to a mean of 743 and 524 μg carbaryl when using hose-end and hand-held spray apparatus, respectively. Greater than 99% of exposure was via the ungloved hands. When normalised for bodyweight and the amount of active constituent used, carbaryl exposure was 0.6 and 0.8 μg/kg bw/g applied, with the respective sprayer types (Merricks, 1998).

Use of a 0.1% ready-to-use liquid, which was applied directly from its pump bottle package, resulted in a mean exposure to carbaryl of 87 μg. Gloves effected a 95% reduction in dermal exposure to the active constituent. When adjusted for bodyweight and the amount of active constituent used, carbaryl exposure was 1.2 μg/kg bw/g applied if gloves were not worn, and 0.06 μg/kg bw/g if applied with gloves (Merricks, 1997b).

When ungloved volunteers treated vegetables with a 9.8% dust product, the mean exposure to carbaryl was 1181 μg. When normalised for volunteer bodyweight and the amount of active constituent used, carbaryl exposure was 2.1 μg/kg bw/g applied (Merricks, 1997b).

1.3 DISCUSSION

1.3.1 Metabolism and Toxicokinetics

The absorption, excretion and toxicokinetics of carbaryl are typical of the carbamate class. Carbaryl is extensively absorbed by the oral route and excreted rapidly in the urine by humans and experimental animals except dogs, in which the faeces is also a significant route of excretion. There is little tendency for carbaryl or its metabolites to accumulate in body tissues, even after subchronic administration. Carbaryl induces the hepatic mixed function oxidase system in mice, showing an induction profile similar to phenobarbital.

In studies previously evaluated by OCS, rats metabolised carbaryl by three main pathways: hydrolysis, alkyl oxidation and arene oxide formation. The latter pathway is believed to proceed via production of epoxide intermediates which are then conjugated by glutathione, either immediately or following the action of epoxide hydrase. There is some evidence (Totis, 1996) that in rats, activity of the arene oxide/epoxidation pathway is enhanced by prolonged dietary administration of 7500 ppm carbaryl, by comparison with the pathway's activity at lower doses. There was a concomitant decline in metabolism via hydrolysis at 7500 ppm. The sponsors suggest that generation of the putative epoxides is associated with formation of kidney, urinary bladder and thyroid tumours in rats receiving 7500 ppm carbaryl during the 2-yr study by Hamada (1993a). In a discussion paper, Cohen (1995) agrees with the registrant's position that epoxidised metabolites of carbaryl cause renal and urinary bladder tumours in rodents. He considers it likely that the bladder tumours observed in rats at 7500 ppm resulted from a direct mitogenic effect on the urinary epithelium, based on his (1994) mechanistic study with propoxur, which has also been shown to cause urinary bladder cancer in rats. Cohen also attributes the proliferative lesions seen in the male rat kidney at 7500 ppm, to mitogenic stimulus.

In the current submission, the sponsors have directed their efforts towards finding a relationship between carbaryl metabolism and carcinogenicity in mice. In Hamada's (1993b) chronic study, vascular, renal and hepatic tumours were increased in mice treated at 8000 ppm, and vascular tumours were also elevated in 1000 and 100 ppm males. With the addition of a 10 ppm group, these same dietary carbaryl levels were administered to mice for 14 d prior to a 50 mg/kg bw oral bolus dose and subsequent quantification/identification of urinary metabolites (Valles, 1999). Consistent with results obtained in rats, pre-treatment with 8000 ppm carbaryl (but not lower doses) increased the urinary excretion of metabolites formed via epoxides, relative to products of hydrolysis and alkyl oxidation. However, the response was smaller in mice than rats. The alkyl oxidation/epoxidation pathway was not identical in the two species, giving rise to one metabolite that was unique to rats and another that was detected only in mice. This might explain the differential response of mice and rats with regard to formation of vascular or renal tumours (which were confined to mice) and neoplasms of the thyroid or urinary bladder (which occurred only in rats).

Although 10 and 100 ppm mice showed a modest decline in the proportion of carbaryl metabolised by hydrolysis (see Table on p 43), the relative activity of the hydrolysis pathway was not reduced at higher doses. Even if the finding did not arise from experimental variation, it is difficult to conceive how it would have any bearing on tumour development.

If the entire body of knowledge about carbaryl metabolism in rats and mice is considered in relationship to tumour formation in these species, some limited conclusions may be drawn, as follows:

- Arene oxide formation/epoxidation occurs in both mice and rats at *all* the carbaryl doses tested;

- The arene oxide/epoxidation pathway becomes relatively more active at 7500 to 8000 ppm, which exceeds the MTD in both mice and rats;

- The (less toxic?) hydrolysis and hydroxylation pathways of carbaryl metabolism may become saturated at dietary levels exceeding 1000 ppm;

- At 8000 ppm, the occurrence of hepatic and/or vascular tumours in female mice and increased incidence of renal and/or vascular tumours among males may indeed occur in response to enhanced epoxide formation; but

- Vascular tumours formed in male mice at 100 and 1000 ppm carbaryl cannot be explained in terms of preferential arene oxidation/epoxidation at 8000 ppm; and

- Since the arene oxide/epoxidation pathway is also active in male mice at 10 ppm (a feeding level not tested in mouse carcinogenicity studies), the findings fail to suggest any particular threshold dose below which the formation of vascular tumours would not occur.

Overall, an association between epoxide formation and tumour development is considered biologically credible, but remains unproven. Beyond showing a difference in the epoxidised metabolites excreted by males of the two species, the metabolism studies have provided no detailed explanation as to why epoxide generation may cause vascular tumours in mice but not rats. It also remains unknown why female mice are more resistant to vascular tumour formation than males. Comparative metabolism data in female mice would have been valuable in this regard. Given that carbaryl metabolism is qualitatively similar in laboratory animals and humans, the current findings in rodents may be relevant to man, but the metabolism data alone cannot be used to predict whether humans would be more or less sensitive to vascular tumourigenesis than mice and rats.

1.3.2 Cholinesterase Inhibition

Carbaryl possesses anticholinesterase activity typical of members of the carbamate class. In rats, ChE inhibition reaches its maximum between 0.5 and 1 h following carbaryl administration by gavage. The subsequent time course of ChE inhibition is both dose- and tissue/site-dependent. Recovery of plasma and RBC ChE activity is rapid (within 2 h post-dosing at 10 mg/kg bw, and within 24 h at 50 mg/kg bw). Brain ChE activity is slower to recover, taking 24 h to fully regain baseline values at 10 and 50 mg/kg bw. At higher doses, reversibility is more prolonged.

ChE inhibition was the main toxicological finding in the newly-submitted 12-month dog study by Hamada (1987), in which there was statistically and biologically significant inhibition of plasma and brain ChE activity at the lowest dietary level of 125 ppm (3.8 mg/kg bw/d). RBC ChE activity was inhibited at and above 400 ppm (11 mg/kg bw/d). Plasma ChE

inhibition was present from wk 5 onwards and persisted until termination, although the effect was diminished at wk 26 and 52, perhaps because of a gradual reduction in achieved carbaryl dose during the study.

By contrast, when a 5-wk dietary study in dogs was performed at the same laboratory 4 yr later, there was no effect on brain ChE activity at the highest feeding level of 125 ppm, and the effect on plasma ChE activity, although present, probably lay near the threshold of biological significance. There were no significant methodological differences between the 5-wk and 1-yr studies. The delivered doses at 125 ppm in the 5-wk study were very similar to those achieved during the first 5 wk of the 1-yr study. Blood samples for ChE assay were obtained approximately 2 h after withdrawal of feed in both studies. It is considered that in the 5-wk study, a combination of biological variation, technical variation in the ChE assay, and lack of statistical power due to small sample size (n=6/group) may have obscured inhibition of plasma ChE at 125 ppm.

Plasma and whole blood ChE have been measured in a human study following single oral doses of up to 2.0 mg/kg bw, and at weekly intervals during administration of repeated oral doses of 0.06 or 0.13 mg/kg bw/d for 6 wk (Wills *et al*., 1968). No inhibition of ChE activity was observed. However, the study is considered unreliable due to a lack of methodological detail and indications from a case report (Hayes & Laws, 1991) that acute ChE poisoning can occur in humans at 2.8 mg/kg bw.

In the Table below, NOELs are presented for plasma, erythrocyte and brain ChE activity. The data suggests that rats and dogs are more susceptible than mice to plasma ChE inhibition.

Also noteworthy is the striking disparity between NOELs demonstrated in the chronic rodent studies compared with those in the acute, 13-wk and developmental neurotoxicity studies, in which the LOELs in plasma, RBC and brain were 10 mg/kg bw/d. Differences between the dosage and sampling regimes employed in the rodent 2-yr and acute and repeat-dose studies are likely to be responsible. Dietary administration was used for the 2-yr experiments, and the rats were probably sampled some hours after cessation of feeding, after the time of peak effect. By contrast, rats in the acute and repeat-dose studies were gavaged and then sampled 1 h post-treatment, at the time of maximum effect. Toxicokinetic differences between dietary and oral bolus dosing may also have contributed to the apparently greater sensitivity of rats in the 13-wk and developmental neurotoxicity studies.

Summary of Doses (mg/kg bw or mg/kg bw/d) at which No Inhibition of ChE Activity Following Carbaryl Administration was seen

Species	Duration	Plasma ChE	Erythrocyte ChE	Brain ChE
Mouse	2 yr	1350	16	16
Rat	Single gavage dose	Not established#	Not established#	Not established#
Rat	13 wk	1	1	1
Rat	25 d (GD 6 – LD10)	1	1	1
Rat	2 yr	70	11	11
Dog	5 wk	1.4	3.8	3.8
Dog	1 yr	Not established*	3.8	Not established*

#ChE inhibition occurred at the lowest dose of 10 mg/kg bw.
*ChE inhibition occurred at the lowest dose of 3.8 mg/kg bw/d.
ND=no data

Note: with the exception of the two studies in dogs, the tabulated studies have been evaluated previously and do not appear in this report.

1.3.3 Neurotoxicity and Behavioural Studies

The effects of carbaryl on the nervous system of rats, chickens, monkeys and humans are primarily related to ChE inhibition and are usually transitory. The EHC Monograph on carbaryl (WHO, 1994) notes disruption to learning in rats treated for 50 d at oral doses of 10-20 mg/kg bw/d, reversible leg weakness in chickens given high doses of carbaryl, but no evidence of demyelination in the brain, sciatic nerve or spinal cord sections in the birds or in long term rodent studies. In a 10-wk study in pigs, dietary administration of carbaryl at 150 mg/kg bw/d caused progressive myasthenia, incoordination ataxia, tremor, muscular contraction, terminal paraplegia and myodegeneration of the skeletal muscle. In the myelinated tracts of the cerebellum, brain stem and upper spinal cord, moderate to severe oedema was associated with vascular degeneration, but no demyelination of nerve tissue was observed.

Few of the neurotoxicity studies on carbaryl that were available before 1995 appear to have been assessed in Australia. However, this situation was improved during the late 1990s by submission of a series of excellent modern studies in rats, which thoroughly characterised the test compound's effects on the central and peripheral nervous systems, ChE activity, behaviour and foetal development. Single gavage doses of 30-50 to 90-125 mg/kg bw caused overt signs of carbamate poisoning and functional deficits in behaviour together with brain, plasma and RBC ChE depression that reversed within 24 to 48 h. ChE depression also occurred following a 10 mg/kg bw dose, but was associated only with reduced motor activity. The NOEL in the 13-wk neurotoxicity study was 1 mg/kg bw/d, based on blood and brain ChE depression and behavioural effects at higher doses. A maternal NOEL of 1 mg/kg bw/d was also established on the basis of these same effects in the developmental neurotoxicity study, but carbaryl had no adverse effects on foetal or pup survival, growth or development at up to and including the highest dose of 10 mg/kg bw/d. In both the subchronic and developmental studies, no adverse findings were made with respect to neuropathology in the adults or offspring.

Supplementary neurotoxicity studies were submitted for inclusion in the current Review. These comprised additional morphometric measurements of the brain in offspring from rat dams treated at the highest dose in the developmental neurotoxicity study discussed above. The supplementary measurements were prompted by a US EPA assessment of that study, which was considered to have demonstrated possibly treatment-related effects on brain weight and morphology at 10 mg/kg bw/d. By contrast, the OCS evaluator attributed the findings to biological variation. The supplementary studies showed no treatment-related differences between the high dose (10 mg/kg bw/d) and control groups, and have no effect on OCS's previous assessment.

1.3.4 Genotoxicity

No further genotoxicity studies have been provided since the 1998 OCS evaluation. Carbaryl has been tested *in vitro* and *in vivo* in bacterial, insect, yeast, plant and mammalian systems. Previous reviews of the genotoxic potential of carbaryl have concluded that carbaryl does not damage DNA and is unlikely to be mutagenic in humans. While carbaryl has demonstrated some clastogenic potential and activity by other endpoints *in vitro* (mitotic recombination, gene conversion, unscheduled DNA synthesis in *Haemophilus influenzae, Bacillus subtilis, Saccharomyces cerevisiae, Aspergillus nidulans*, human lymphocytes and rat hepatocytes) at high doses that produced marked cell toxicity, it is not an *in vivo* clastogen. Carbaryl has yielded negative results in all but 2 mutagenicity assays in bacteria, and although several mutagenicity assays have been conducted in cultured mammalian cells, only one equivocally positive result has been obtained.

However, it would be premature to rule out the possibility that genotoxicity can be mediated by the (hypothetical) epoxides generated during carbaryl metabolism. If these entities are indeed formed in sufficient quantities, they may react with genetic material in some or all of the target tissues, under conditions that are not duplicated by the test protocols employed so far. The question will probably remain unanswered until attempts are made to detect molecular adducts on chromosomal material or other intracellular macromolecules from within the liver, kidney, thyroid, urinary bladder and hepatic/splenic vascular system of mice and rats receiving carbaryl.

1.3.5 Reproduction and Development

One of the most significant deficiencies in the carbaryl database has been the lack of modern reproduction and developmental studies. This limitation has now been addressed with the submission of a new 2-generation reproduction study in rats and developmental studies in rats and rabbits, which were performed in accordance with current GLP standards and Test Guidelines.

The 1996 JMPR observed that the (then) available reproduction studies with carbaryl were deficient by contemporary standards. In previous 3-generation studies in rats, fertility was impaired and post-natal survival and growth were reduced at dietary doses >2000 ppm (equal to 125 mg/kg bw/d) but a dose of 100 mg/kg bw/d did not induce maternal toxicity. When carbaryl was administered by gavage, maternal toxicity was not observed at 25 mg/kg bw/d but maternal toxicity, reduced litter size and viability were found at 100 mg/kg bw/d. The Meeting recommended that a new 2-generation study be carried out in rats, with special attention to the male reproductive system, upon which effects had been observed in some long term toxicity studies by gavage at doses significantly lower than those evaluated in dietary studies.

OCS was highly supportive of this recommendation, having previously evaluated a published paper (Pant *et al.*, 1995) showing disrupted testicular morphology and spermatogenesis in Wistar rats gavaged for 90 d (5d/wk) at 50 or 100 mg/kg bw/d. Pant *et al.* observed testicular congestion and oedema, moderate atrophy of the seminiferous tubules, an approximately 2-fold increase in the proportion of abnormal sperm and a 40% reduction in sperm count *vs.* control at 50 mg/kg bw/d. At 100 mg/kg bw/d, testicular congestion and oedema were more intense, masses were present within the seminiferous tubules, the proportion of abnormal

sperm was trebled and sperm count was depressed by 60%. There were dose-related depressions in glucose 6-phosphate and sorbitol dehydrogenase activity and elevations in LDH and GGT activity. The findings of Pant *et al*. are not isolated. Vashakidze (1975; evaluated by WHO in EHC 153 [1994]) reported decreased sperm motility and increased sperm abnormalities in rats intubated orally with carbaryl for 1 mo at doses of 5 mg/kg bw/d or greater.

The current 2-generation reproduction study (Tyl *et al.*, 2001) found possible treatment-related effects on the male reproductive system at the highest dose of 1500 ppm (approximately 97 mg/kg bw/d). A single F0 adult male was found to be producing 100% non-motile sperm that lacked tails. The finding was not correlated with reproductive failure, however, as the animal had mated and conceived a litter of viable pups. (Given that 3 wk had elapsed between mating and sacrifice of the F0 males, complete loss of active sperm may have not developed until the post-mating period). There was also an apparent dose-related increase in percentage abnormal sperm seen in the 1500 and 300 ppm F0 males, but it was not repeated in the F1 generation.

Differences in dosing methods may explain the variation between the outcome of the studies by Tyl *et al.* and Pant *et al*. The 1500 ppm males in the study of Tyl *et al.* received carbaryl by dietary administration, whereas Pant *et al.* administered carbaryl orally in peanut oil (the exact technique was not described). Oral absorption of carbaryl is rapid, suggesting that higher peak blood and tissue levels may be attained after bolus dosing than following dietary intake of an equivalent dose over the usual 8-h rodent feeding period. There may also be genetic differences in susceptibility between the Wistar rats used by Pant *et al.* and the CD (Sprague-Dawley) strain used by Tyl *et al* Unfortunately, there is insufficient information available on the study by Vashakidze (1975) to enable comparison between the test material, animals and methods used by that author and Tyl *et al*.

The delayed puberty in F1 pups and reduced anogenital distance at birth in F2 males at 1500 ppm raise the question as to whether carbaryl mediates a specific effect on sexual development. However, the study's findings do not support such a hypothesis. Anogenital distance was found to be dependent on pup birth weight (*ie*, smaller male pups tended to have shorter anogenital distance) and hence is unlikely to have been reduced by feminised sexual development of male foetuses. Puberty was retarded in both sexes (which provides further evidence against a gender-specific effect) and retardation occurred only in the presence of inhibited bw gain. These findings are therefore both considered to be secondary to effects on bw and bw gain.

In the developmental studies, maternotoxicity was seen as cholinergic signs in rats, inhibition of plasma and RBC ChE activity in rabbits, and depressed weight gain in both species. Foetal development was retarded at maternally toxic doses, but there were no treatment-related visceral anomalies or malformations. The results were broadly consistent with those of studies previously evaluated by the OCS and the JMPR.

1.3.6 Carcinogenicity

Carbaryl is remarkable for its carcinogenic activity in the chronic rodent studies by Hamada (1993a and 1993b), having caused tumours of the thyroid, urinary bladder and liver in rats, and kidney, liver and vascular system in mice. In the current submission, cell cycling studies on tissue specimens from Hamada's studies (with the exception of sites of vascular tumour

formation) demonstrated enhanced cellular division in target tissues. These results were in marked contrast to negative findings in a series of previous oncogenicity studies in both species, dating to the early 1960s.

However, with the exception of vascular tumours, carcinogenicity did not occur below the highest doses administered (8000 and 7500 ppm in diet to mice and rats, respectively). In many respects, the high dose tumours are suggestive of inappropriate study design. On a daily basis, the high dose groups received equivalent to or greater than the acute oral LD50, and displayed marked systemic toxicity including depressed weight gain and feed consumption, behavioural changes, cataracts and anaemia. Thus, the 7500/8000 ppm groups were treated at above the maximum tolerated dose. Since carbaryl has not shown any convincing evidence of genotoxic activity, and because NOELs of 1000 and 1500 ppm were demonstrated in the respective species for bladder, hepatic, thyroid and renal tumours, neither OCS nor the ACPH has regarded these high dose tumours as a barrier to continuing registration of carbaryl, subject to adequate safeguards that would limit public exposure to the chemical.

From a regulatory standpoint, the vascular tumours are of significantly greater concern. Although these did not develop in female mice at below the 8000 ppm feeding level, there was no apparent NOEL in males, even at the lowest dose of 100 ppm (equivalent to approximately 16 mg/kg bw/d). In the opinion of the OCS, ACPH and JMPR, historical control data on the incidence of vascular tumours has failed to demonstrate that Hamada's findings were attributable to biological variation. Furthermore, while there are often fairly well established non-genotoxic modes of action underlying the development of liver, thyroid, kidney and urinary bladder tumours in rodents, vascular hemangioma and hemangiosarcoma are more difficult to explain, and their human relevance cannot be dismissed.

The sponsors have now focussed on eliminating genotoxicity as a probable mode of action for carbaryl. This has been attempted by use of a novel short term carcinogenicity bioassay in male p53 "knockout" mice, which compared tumour development among animals treated with carbaryl, d-limonene (as a negative control) and urethane (as a positive control).

The function of the p53 gene is related to regulation of the cell cycle. Cellular levels of p53, a phosphoprotein transcription factor, are greatly increased by radiation and other DNA damaging agents, and this increase in p53 is accompanied by an arrest in late G1 of the cell cycle. Wild type p53 can also mediate apoptosis (Donehower, 1996). By contrast, cells in which the p53 gene is deficient may continue to replicate while incorporating genetic errors that would normally be repaired or excised. Many types of human tumours contain mutations and loss of the p53 gene. In addition, germ line mutations in p53 have been identified in affected individuals of Li-Fraumeni syndrome families, who have a 50% likelihood of developing cancer by the age of 30 (Donehower, 1996).

The heterozygous p53-deficient mice used in the current oncogenicity study with carbaryl, are phenotypically normal but have enhanced susceptibility to genotoxic events, both spontaneous and induced. The pattern of spontaneous tumour formation among p53 heterozygous mice is of major importance to their utility in the investigation of vascular tumours. Less than 8% of these mice develop tumours before 9 months of age, but tumour incidence subsequently increases to 50% by 18 months and 90% by 2 years. The principal spontaneous neoplasia in p53 +/- animals are soft tissue sarcomas, osteosarcomas and lymphomas (approximately 30% incidence, each), with brain tumours and unspecified

carcinomas accounting for the remainder (Donehower, 1996). Vascular hemangiomas and hemangiosarcomas are uncommon, which does enhance the biological significance of their formation when p53 knockout mice are treated with xenobiotics.

Donehower (1996) notes accelerated development of liver hemangiosarcoma in dimethyl nitrosamine treated p53 +/- mice, while in the current study in p53 knockout mice, vascular tumours were induced by the genotoxic carcinogen, urethane (Bigot, 1999). It is of interest that urethane is metabolised to vinyl carbamate, which is further metabolised to the ultimate carcinogen, vinyl carbamate epoxide. Vinyl carbamate epoxide reacts with DNA to form one minor and two major adducts, giving rise to an A to T transversion mutation (Bowden, 1997).

Detoxification of epoxides is essential for cell survival and depends mainly on the action of epoxide hydrase or glutathione transferase. Hayes (1994) notes the existence of two forms of epoxide hydrase; an endoplasmic reticular form highly active in adult rats (especially males), and a cell cytosol form that is more active in mice than rats. Perhaps sensitivity to vascular tumour formation can be influenced by species- and gender-specific differences in epoxide hydrase activity. Circumstances leading to glutathione depletion may also enhance the vulnerability of target cells to electrophilic injury.

No treatment-related tumourigenesis occurred in p53 heterozygous mice treated with d-limonene, a non-genotoxic renal carcinogen in male rats that acts by causing α_{2U}-globulin accumulation. Nor did carbaryl elicit tumourigenesis, at up to the highest dietary feeding level of 4000 ppm.

Taken at face value, the negative findings with carbaryl in p53-deficient mice provide support for the view that carbaryl need not be regulated as a genotoxic carcinogen. Nevertheless, any chemical metabolised via a reactive electrophile must be viewed with concern.

Despite the knowledge gained from the current studies, there are still limitations in our understanding of carbaryl's carcinogenic properties, and its mode or mechanism(s) of action remain uncharacterised. The submitted cell cycling studies did not examine vascular tissue. There is a lack of regulatory experience with p53 knockout mouse carcinogenicity studies, which is sufficient to prevent OCS from discounting the results obtained in Hamada's (1993a) conventional 2-yr experiment. There is also no indication as to which of the three modern carcinogenicity bioassays with carbaryl (6-month "knockout" mouse, 2-yr mouse or 2-yr rat) has the most human relevance. Under the circumstances, the reviewer considers that OCS should continue to uphold use of an enhanced safety factor and reduce public exposure to the lowest extent reasonably achievable.

1.3.7 Human Studies

So far, there is no evidence that carbaryl is carcinogenic in humans. An epidemiology study of workers employed at a US plant that produces carbaryl showed a slightly lower overall rate of mortality from cancer than expected from the general population. Although there was an excess of brain tumours, this lay well within the range of chance and cannot be attributed to exposure to carbaryl.

The current submission included human exposure studies which measured the amount of carbaryl deposited on the skin and clothing of volunteers who were using American carbaryl products in simulated home garden and home veterinary situations. The concentration of

carbaryl in their breathing zone air was also measured. The studies were noteworthy for their good design and clear description of the activities performed by the volunteers, and yielded detailed data on the extent and pattern of carbaryl exposure, the amount of inter-individual variation in exposure, and the effectiveness of gloves and clothing in reducing exposure.

The product that had by far the greatest potential for human exposure was a 5% carbaryl veterinary dusting powder. Then, in decreasing order of exposure potential, were 10% vegetable dusts, a 22% liquid concentrate applied to vegetables or trees by spray, and a 0.1% ready to use vegetable spray. In all cases, the majority of exposure occurred via the hands. The veterinary dusting powder also caused significant exposure by inhalation whereas inhalation exposure by vegetable dusting and application of carbaryl sprays was negligible. In general, only about 5% or less of carbaryl that became deposited on the external clothing penetrated to the skin, and comparison between gloved and un-gloved subjects showed that gloves effected a 95% reduction in exposure to the active constituent.

There was wide inter-individual variability in the extent of exposure to carbaryl after using the same product for the same application. For example, after applying insecticidal dust to dogs, the most carbaryl found on a volunteer's internal dosimeter was 13,153 μg, compared with a minimum of 63 μg. When spraying vegetables without gloves, the lowest and highest carbaryl loads on the hands were 63 and 4,440 μg, respectively. This occurred despite all members of the study group performing standardised tasks under supervision, which would have prevented them from preparing grossly over or under strength spray mixtures, or mis-applying the various products.

In Section 10 of this review, the American exposure studies have been used as the basis of estimating the exposure and systemic dosing of persons using Australian carbaryl products.

1.3.8 NOEL Considerations

A summary of the NOELs determined for carbaryl are shown in the Table below. Note that the Table omits studies that have not been evaluated by OCS, are unsuitable for regulatory purposes, or have been superseded by replacement data.

Study	NOEL (mg/kg bw/d)	LOEL and Toxic Effects
Dogs: 5-wk dietary	1.4	3.8 mg/kg bw/d: depressed plasma ChE activity
Mice: 6-month dietary	5.2	17.5 mg/kg bw/d: deposits in urinary bladder epithelium.
Mice: 2-yr dietary	Not established	16 mg/kg bw/d: vascular system tumours in males.
Rats: 2-yr dietary	11	70 mg/kg bw/d: depressed bw gain and brain and RBC ChE activity.
Dogs: 1-yr dietary	Not established	3.8 mg/kg bw/d: depressed plasma and brain ChE activity
Rats: 2-generation dietary reproduction	4.7	19 mg/kg bw/d: decreased parental bw gain, bw, feed consumption and conversion together with increased pup mortality during lactation.

Study	NOEL (mg/kg bw/d)	LOEL and Toxic Effects
Rats (male): 90-d reproduction by gavage	Not established	50 mg/kg bw/d: lethargy, decreased bw gain and spermatogenesis, increased testicular LDH and GGT activity, testicular atrophy.
Mice: dietary developmental	No adverse effects at highest dose of 30 mg/kg bw/d	-
Rats: developmental by gavage	4.0 for both maternal and foetal effects	30 mg/kg bw/d: salivation, depressed feed consumption and bw gain in dams; reduced bw and delayed ossification in foetuses.
Guinea pigs: developmental by gavage and dietary administration	No treatment-related effects at highest doses of 200 mg/kg bw/d (gavage) or 300 mg/kg bw/d (dietary)	-
Rabbits: developmental by gavage	5.0 for maternal effects 50 for foetal effects	Does: 50 mg/kg bw/d: plasma and RBC ChE inhibition; Foetuses: 150 mg/kg bw/d: depressed bw.
Dogs: dietary developmental	3.1 for foetal effects No maternal effects at highest dose of 50 mg/kg bw/d	Pups: 6.3 mg/kg bw/d: skeletal and visceral abnormalities in the absence of maternal toxicity.
Rats: 13-week neurotoxicity by gavage	1.0	10 mg/kg bw/d: blood and brain ChE inhibition and reduced motor activity.
Rats: developmental neurotoxicity by gavage	1.0 for maternal effects No adverse effects on pups	10 mg/kg bw/d: decreased maternal bw gain, ataxia, gait disturbance, tremor, constricted pupils, inhibited plasma, RBC and whole blood ChE activity.

1.3.9 Determination of Public Health Standards

Acceptable Daily Intake

In October 1998 the ACPH reconsidered the ADI for carbaryl, in light of the expanded toxicological database then available, and the draft NHMRC (1999) guidelines for derivation of modifying factors for seriousness of carcinogenic effect. The ACPH recommended that a 4000-fold safety factor be applied to the LOEL of 100 ppm (16 mg/kg bw/d) for vascular tumours in male mice in a 2-yr dietary study, giving a revised ADI of 0.004 mg/kg bw/d.

At its October 2000 meeting, the ACPH re-considered the ADI for carbaryl in light of the reviewed carcinogenicity study in p53 "knockout" mice and supplementary studies on the mechanism of tumour formation. The committee confirmed that the continued use of the 4000-fold safety factor for deriving the ADI remained appropriate given the continuing

limitations in understanding carbaryl's carcinogenicity in rodents. No new data relevant to the carcinogenicity of carbaryl have subsequently become available. Furthermore, none of the additional studies evaluated in this report is considered to be a more suitable basis for the ADI than the current pivotal 2-yr study in mice.

However, OCS notes that following the JMPR-2001 consideration of the carcinogenicity study with carbaryl in p53 "knockout" mice, the JMPR ADI for carbaryl has been increased from 0.003 to 0.008 mg/kg bw/d. This was brought about by reducing the safety factor applied to the 100 ppm LOEL for vascular tumourigenesis, from 5000- to 2000-fold. Furthermore, comment received from the data provider on the (June 2002) draft of the Australian review has highlighted the conservatism of Australia's 4000-fold safety factor, particularly in light of the negative result obtained with carbaryl in the study in P53-deficient mice.

Taking all relevant factors into consideration, the OCS agrees that the negative result obtained in the 6-month study in P53-deficient mice has indeed significantly increased the weight of evidence that carbaryl is not genotoxic *in vivo*, thereby reducing concern over potential effects on human health. This enables the component for "confidence that carbaryl is genotoxic" to be reduced from 2 to 1, and using the NHMRC criteria on deriving safety factors, the effect of the modification is to reduce the overall safety factor from 4000 to 2000. Application of the 2000-fold safety factor to the LOEL of 100 ppm (16 mg/kg bw/d) for vascular tumours in male mice yields a revised ADI value of 0.008 mg/kg bw/d.

This approach yields the same outcome as the conventional method of deriving safety factors for agricultural and veterinary chemicals, which would incorporate the standard 100-fold component (10 for extrapolation from animals to humans, X 10 for variation in sensitivity within the human population), together with an additional 10-fold factor for use of a LOEL instead of a NOEL, and an extra 2-fold factor allowing for the remaining uncertainty as to the mode and mechanism of vascular tumour formation and for the impossibility of discounting the relevance of vascular tumours to humans.

Given that the LOEL for vascular tumour formation is probably near the threshold dose for tumourigenesis in mice, a margin of greater than 2000-fold between the ADI and the LOEL would not be likely to increase human safety. Hence, a 2000-fold SF should be sufficient to prevent a carcinogenic hazard to humans from dietary intake.

Acute Reference Dose

At present, there is no Australian ARfD value for carbaryl. Among the toxicological studies that would possibly be a suitable basis for an ARfD, the lowest NOEL is 0.06 mg/kg bw/d, established in a 6-wk oral study performed in male prisoners (Wills *et al.*, 1968). An increased urinary amino acid:creatinine ratio was observed at the next highest dose of 0.12 mg/kg bw/d, and was interpreted by the JMPR (1970) as a slight decrease in the ability of the proximal convoluted tubule to re-absorb amino acids. Plasma and whole blood ChE activity was unaffected at either dose. Clinical signs or effects on ChE activity were not observed in a preliminary range-finding experiment, in which pairs of prisoners received single oral doses of up to 2 mg/kg bw carbaryl. However, the study authors failed to specify the time interval elapsed between dosing and blood sampling during the main and range-finding experiments. It is therefore possible that undetected ChE inhibition occurred, given that ChE activity recovers rapidly following inhibition by carbaryl. Although the prison pharmacist checked

the subjects' mouths after dosing to ensure the capsules had been swallowed, there must also be some uncertainty as to whether the carbaryl was indeed taken as intended by the study authors. Given these uncertainties, the Wills *et al.* study is considered unsuitable for regulatory purposes.

Two case studies of adverse effects in humans following carbaryl ingestion are reported in the *Handbook of Pesticide Toxicology* (Hayes & Laws, 1991). A scientist exploring the possible value of carbaryl as an anthelmintic ingested 250 mg (approximately 2.8 mg/kg bw). After 20 min, he suddenly experienced violent epigastric pain, and a little later he began to sweat profusely. A 1 mg dose of atropine produced little improvement. He gradually developed great lassitude and vomited twice. One h after taking the carbaryl, and after a total atropine dose of 3 mg, he felt better, and was completely recovered after 2 h. In the second incident, a scientist ingested (on an empty stomach) a suspension containing about 420 mg carbaryl (approximately 5.5 mg/kg bw). (He had previously taken larger doses about 1 h after a meal without any resulting illness.) After 85 min, he noted a slight change in vision and after 90 min he began to feel nauseated and lightheaded. Two mg atropine provided relief but the symptoms returned. The atropine dose was increased to 4.8 mg. Despite this, the nausea persisted and profound weakness, profuse sweating and hyperperistalsis developed. The symptoms attained maximum severity about 2 h after their onset, but definite improvement occurred within 3 h of onset and recovery was nearly complete after 4 h.

Two additional studies in humans are briefly summarised in the IPCS review of carbaryl. Both studies (Hansen, 1978 and Ward *et al*., 1988) were investigations of carbaryl metabolism and involved administration of oral doses of up to 1 mg/kg bw. No mention was made of any clinical signs or other treatment-related effects in the subjects. Although suitable data on RBC ChE activity in humans could be used for setting an ARfD for carbamates or OPs, the study of Wills *et al*. is considered unreliable, and there are no other data that would establish NOELs or LOELs for ChE inhibition by carbaryl in humans. The ARfD for carbaryl therefore has to be based upon studies in experimental animals.

A series of acute dose rangefinding studies was performed in unfasted rats gavaged with carbaryl at 10 mg/kg bw and above. The 10 mg/kg bw dose did not cause clinical signs but elicited a transient 40% decrease in motor activity (in males) at 1 h post-treatment, together with a 1 °C depression in body temperature (in females). Plasma and RBC ChE activity were depressed by up to approximately 30%. Brain ChE activity was inhibited by 30-50%.

The 1-yr dog study evaluated here did not demonstrate a NOEL, due to brain and plasma ChE inhibition at the lowest dietary dose of 3.8 mg/kg bw/d. RBC ChE activity was not affected at that dose, but was inhibited at and above 11 mg/kg bw/d. Despite these findings, clinical signs did not occur even at the highest dose of 34 mg/kg bw/d. The 5-wk dog study found no effect on RBC or brain ChE activity at the highest dose of 3.8 mg/kg bw/d, and formed the basis of the ARfD set by JMPR in 2001. In 1996, the JMPR summarised the Hayes and Laws case report in humans in which clinical signs were observed at 2.8 mg/kg bw. However, in the 2001 Report there was no comment about this observation. Given that overt toxicity in humans occurs *below* the NOEL for RBC and brain ChE inhibition in dogs, dogs must be significantly more resistant to the effects of carbaryl than humans. In the absence of comparative data on the toxicokinetic and toxicodynamic behaviour of carbaryl in dogs and humans, there is no explanation for the inter-species difference in sensitivity. Hence, the NOEL in the 5-wk dog study can not be used as a basis for the ARfD, because it corresponds to an effect level in humans.

The lowest NOEL in repeat-dose studies in animals which is also not associated with clinical signs in humans is 1 mg/kg bw/d, established in rat 13-wk subchronic and developmental neurotoxicity studies, based on behavioural indications of autonomic neurotoxicity and brain, plasma and erythrocyte ChE depression (LOEL=10 mg/kg bw/d). Application of a 100-fold safety factor to the 1 mg/kg bw/d NOEL would yield an ARfD of 0.01 mg/kg bw.

1.3.10 Public Exposure

Carbaryl is used for the control of a diverse range of insect pests on animals and edible and ornamental plants, and is also effective against other arthropods including millipedes when applied to and around buildings. Public exposure to carbaryl is therefore expected to be widespread, occurring from:

- Consumption of residue in commercially treated fruit, vegetables and other commodities;
- Consumption of residue in home grown fruit and vegetables;
- Dermal and inhalational exposure when preparing and/or using HG and HV products;
- Dermal contact with pets, carpets, lawns and exterior surfaces treated with HG/HV products; and
- Dermal contact with surfaces treated by pest control operators.

The pattern of public exposure from domestic use is likely to be discontinuous, given seasonal variation in plant growth, fruit or vegetable production and pest activity, including fleas. However, there is scope for repeated use of carbaryl products in and around the home during the warmer months of the year. Re-treatment intervals vary between products. Pet ectoparasiticidal dusts and shampoos are applied every 1–2 wk, while ear drops are administered twice daily for at least 14 d. Vegetables require treatment each 7–10 d and recommended re-treatment intervals for fruit are 2–3 wk. Lawn treatments are applied monthly.

1.3.11 Acute Toxicity

The APVMA "Guidelines for pesticides used by householders" (Appendix 3-1 of the "Guidelines for registering agricultural chemicals") stipulate that any domestic pesticide formulation that may be ingested should not be expected to be acutely toxic to a child at doses up to 1500 mg/kg bw, and should not be acutely toxic at dermal doses of up to 1000 mg/kg bw. The irritancy to skin and eye of domestic pesticide formulations should be low.

During preparation of this report, it was noted that several products currently sold in HG pack sizes are unlikely to comply with the above conditions. These products are wettable powders containing 800 g/kg carbaryl, and liquids containing 400 and 500 g/L carbaryl. As none of the available OCS toxicological evaluations contain assessments of acute studies on the actual carbaryl-based products, their toxicity has to be estimated by extrapolation from the characteristics of the active constituent and excipients. Technical grade carbaryl has a worst acute oral LD50 of 246 mg/kg bw in rats, a dermal LD50 >2000 mg/kg bw in rabbits, is slightly irritating to the rabbit eye, but is not a dermal irritant or sensitiser. Whilst there appear to be no problems with excessive irritation or dermal toxicity, the acute oral LD50 of 800 g/kg WPs is estimated at 308 mg/kg bw, and the corresponding values for the 400 and 500 g/L products are predicted to be 616 and 492 mg/kg bw, respectively (see Appendix V). Unless the acute oral hazard were influenced by other formulation constituents, only

formulations containing 160 g/kg or less of carbaryl would have an oral LD50 of 1500 mg/kg bw or greater, and so comply with the guideline.

1.3.12 Exposure from Use of Home Garden and Home Veterinary Products

Potentially some 175,000 domestic units would be sold per year, of which about 50,000 units would be pet shampoos, powders and ear drops. The maximum number of households exposed to carbaryl from HG/HV products would therefore be around 175,000 assuming 1 unit/household/yr. Given that some households will purchase 2 or more carbaryl products annually, a more realistic estimate would be 80-100 000. However, an unknown number of other households will be treated with carbaryl by pest control operators, for which complete data are not available. Overall, if there were 4 persons per household, up to 3-4 hundred thousand individuals could potentially be exposed to carbaryl in HG/HV products, although to a varying extent.

OCS has estimated the systemic doses that would be absorbed by carbaryl product users under typical Australian domestic conditions. Systemic doses were usually estimated by extrapolation from the mean dermal and inhalation exposure measured in American volunteers, adjusting for dermal absorption of carbaryl. Recognising that some American product users were much more heavily exposed than average, another set of systemic dose estimates was prepared by extrapolation from the upper limit of exposure. Variation in the user's clothing was taken into account, with dose estimates being prepared for persons wearing long pants and sleeves, or short pants and sleeves. Scenarios involving either 30 min or 2 h exposure were also considered. Thus, for most of the products examined, there were 8 exposure scenarios, yielding dose estimates that ranged from a mean exposure for 30 minutes wearing long pants and sleeves, up to a worst case exposure for 2 h wearing short pants and sleeves.

Some Australian HG/HV products were found to be potentially capable of delivering systemic doses to users in excess of the ADI for carbaryl, the recommended ARfD, or both, under what are considered to be feasible exposure scenarios. However, 10 g/L pet shampoos, 20 g/kg garden/vegetable dusts, wettable powders and 1 g/L ready-to-use liquid sprays were not likely to deliver a toxicologically significant dose of carbaryl. A condensed summary of the user systemic dose estimates is presented below. For clarity, the Table shows only the combinations of products and exposure scenarios likely to deliver doses above the ADI and/or ARfD. Dose estimates exceeding the ARfD or ADI are highlighted.

Summary table: Exposure scenarios leading to estimated systemic carbaryl doses exceeding the ADI and/or ARfD

Exposure scenario	Duration of dermal exposure (h)	% of ARfD (10 µg/kg/d)	% of NOEL for ChE inhibition (1000 µg/kg/d)	% of ADI (8 µg/kg/d)	% of LOEL for tumours (16000 µg/kg/d)
50 g/kg pet dusts – treatment of 1 medium sized dog					
Top of range exposure, long pants & sleeves	0.5	90	0.9	117	0.058
	2.0	210	2.1	265	0.13

Top of range exposure, short pants & sleeves	0.5 2.0	200 650	2 6.5	253 812	0.13 0.41
50 g/kg pet dusts – treatment of aviary					
Typical exposure, long pants & sleeves	2.0	82	0.82	103	0.051
Top of range exposure, long pants & sleeves	0.5 2.0	190 420	1.9 4.2	233 525	0.12 0.26
Typical exposure, short pants & sleeves	2.0	150	1.5	188	0.094
Top of range exposure, short pants & sleeves	0.5 2.0	400 1300	4 13	500 1625	0.25 0.81
10 g/L ear drops – accidental spill during treatment					
Typical exposure, confined to hand	2.0	125	1.25	157	0.078

Exposure scenario	**Duration of dermal exposure (h)**	**% of ARfD (10 µg/kg/d)**	**% of NOEL for ChE inhibition (1000 µg/kg/d)**	**% of ADI (8 µg/kg/d)**	**% of LOEL for tumours (16000 µg/kg/d)**
50 g/kg dusts – treatment of garden and vegetables					
Top of range exposure, long pants & sleeves	2.0	93	0.93	117	0.059
Top of range exposure, short pants & sleeves	2.0	113	1.13	142	0.071
60 g/L hose on insecticide – treatment of turf					
Top of range exposure, long pants & sleeves	2.0	150	1.5	188	0.094
Top of range exposure, short pants & sleeves	0.5 2.0	120 500	1.2 5	150 619	0.075 0.31

Before examining each group of products, it is necessary to consider the toxicological implications of the dose estimates tabulated above. The maximum dose estimate for use of one of the products (treatment of aviary with 50 g/kg bird dust) is equivalent to 13 times higher than the recommended ARfD (based on a 100-fold safety factor applied to the NOEL for ChE inhibition) and 16 times higher than the ADI (based on a 2000-fold safety factor applied to the LOEL for cancer). From a public health standpoint, this represents an unacceptable erosion of the safety margin for both endpoints, even though the use pattern for Australian carbaryl products suggests that daily exposure to such a high dose is improbable, and the APVMA Adverse Experience Reporting Program has not received any reports of toxicity in persons using carbaryl-based veterinary products.

The question arises as to whether the ARfD or the ADI is the more suitable upper dose limit for persons using HG or HV products. The ARfD for carbaryl provides a 100-fold margin of safety (MoS) for ChE inhibition and behavioural indications of neurotoxicity. However, the ARfD does not take vascular carcinogenesis into account. Because a NOEL for vascular carcinogenesis has **not** been demonstrated, it is impossible to quantify or assure the adequacy of any MoS between the ARfD and vascular cancer. Nor is it possible to dismiss the relevance of vascular cancer to humans in an acute or short-term exposure scenario, especially without a defined mode of action for vascular carcinogenesis. The ADI has been set with an enhanced 2000-fold safety factor on the LOEL, in order to optimise protection against vascular cancer, and it is certain to protect against ChE inhibition (since the ADI is 80% the ARfD).

OCS has therefore formulated its recommendations with a view to constraining the upper limit of carbaryl intake to the ADI, if this can be reasonably achieved by use of label hazard warning statements and directions to wear protective clothing and equipment. Home garden/veterinary products that have the potential to cause carbaryl intake above the ADI under anticipated conditions of use and are not amenable to risk reduction by means of protective hygiene/clothing/equipment, are considered unsuitable for continued registration. Also regarded as unsuitable, are products for which there are insufficient data to estimate the extent of householder exposure.

The most hazardous products are veterinary dusts. From the above Table, it is apparent that applying a 5% pet dust to one dog could deliver systemic doses up to 8-fold higher than the ADI and 6.5-fold greater than the recommended ARfD. Aviary treatment could deliver double these doses. The high level of exposure from pet dusts can be attributed to discharge of the powder into the user's breathing zone air, together with the need to touch the pet while working the powder into its feathers or fur. It is very difficult to protect product users from this combination of dermal and inhalation exposure, other than by requiring them to wear a disposable dust mask, and cover their skin with gloves and long clothing. However, this approach to risk management is unlikely to meet with success if adopted for pet grooming products, as user perception would be negative and compliance improbable. Given that carbaryl shampoos are available and have a lower potential for user exposure than dusts, the most effective course of action would be to withdraw carbaryl based pet dusts and powders from the HV market.

By comparison with pet dusts, the likely extent of user exposure from flea collars and insecticidal shampoos is an order of magnitude lower. Systemic uptake from shampooing one or two dogs is expected to lie below the ADI. Although persons treating 3 or more animals could receive systemic doses exceeding the ADI or ARfD, exposure could easily be reduced

to negligible levels if users wore rubber gloves. Since pet owners are not likely to suffer inconvenience from gloves while washing their animal(s), an appropriate new entry for carbaryl shampoos should be placed in the FAISD Handbook. Systemic exposure from flea collars is probably intermediate between exposure from dusts and shampoos. However, estimates of user exposure are hampered by a lack of data on free carbaryl levels on the surface of flea collars. OCS estimates that unwrapping and fitting a new flea collar could lead to absorption of a systemic dose equivalent to 85% of the ADI, but the delivered dose could exceed the ADI or ARfD if carbaryl accumulates on the collar's surface between manufacture and use. Gloves would provide an ideal level of protection but would impede the user unacceptably. However, exposure can be minimised by directing users to wash their hands. In any case, occasional excursions above the ADI are not necessarily of toxicological concern, and user exposure will be infrequent given that the reference product has a 4-month life. As shown in Section 10 of this report, carbaryl intake from handling a pet treated with a carbaryl shampoo or collar is unlikely to attain the ARfD or ADI.

Although capable of delivering systemic doses up to 6 times higher than the ADI and 5-fold above the recommended ARfD, carbaryl home garden vegetable dusts, wettable powders and liquids would be likely to cause much less user exposure than pet dusts. This is primarily because garden use often entails discharge at or below waist height, and manual contact with treated vegetation is not required. Based on Merricks' (1997b, 1998) evidence, inhalation exposure is negligible, even when spraying trees, and a combination of long pants, long sleeves and gloves is sufficient to limit exposure to levels well below the ADI when dusting or spraying in the home garden.

1.3.13 Post-application Exposure

The use of some carbaryl based home garden and professional products inside domestic homes, on lawns and as a chemical barrier on paths and walls, brings with it the question of occupants' exposure from treated surfaces. In the absence of relevant experimental data, OCS has relied upon US EPA default factors for estimating the transfer of carbaryl from turf and hard surfaces, in conjunction with the application rate per unit area calculated from the product label instructions.

Sitting or lying on treated grass or walking barefoot on treated paving, could deliver systemic doses above the ADI and ARfD if the carbaryl was not washed off the contaminated skin within an hour. Here, OCS believes that the appropriate risk reduction strategy is to direct householders to keep off treated surfaces.

The indoor use of carbaryl is more problematic. The only known indoor application of carbaryl is as an insecticide/deodorant dust for carpets, rugs and animal bedding. Label instructions specify that the dust should be sprinkled lightly and then removed by vacuuming after 1 h. It is impossible to estimate the likely extent of householder exposure because neither the application rate nor the efficiency of vacuuming are available. OCS also has no data on the persistence of carbaryl indoors. Noting that household residents (especially infants) are more likely to make contact with a treated floor than grass or pathways, and that ChE depression has been recorded in persons whose residences have been treated indoors with carbaryl (WHO, 1994), OCS believes that label warnings are insufficient to ensure safety. Consequently, it is recommended that carbaryl should not be registered for indoor use.

1.3.14 Poisons Scheduling

Carbaryl is classified as a Schedule 6 poison in the SUSDP, with Schedule 5 entries for preparations containing 10 per cent or less of carbaryl, or when impregnated into plastic resin material containing 20 per cent or less of carbaryl. Carbaryl preparations for human therapeutic use are listed in Schedule 4, but none are currently on the Australian market.

1.3.15 Committee Considerations

NDPSC

The Poisons Schedule status of carbaryl was considered by the National Drugs and Poisons Schedule Committee at its 36th meeting (15th-17th October 2002). The Committee noted that removal of the Schedule 4 entry had been recommended by the CRIH on the basis that carbaryl was carcinogenic in experimental animals; the available data did not permit an adequate risk assessment to be undertaken in relation to treatment of head lice and there were no registered human therapeutic products containing carbaryl.

However, the Committee considered that:

- Removal of carbaryl from Schedule 4 would delete an important import control over therapeutic goods for human use containing carbaryl, that is, the need for a prescription and the agreement of a physician to the proposed use.

- Likewise removal from Schedule 4 would permit a pharmacist to include carbaryl in a compounded preparation for individual use.

- Under the Trans-Tasman Harmonisation guidelines agreed by the Committee where both NZ and Australia had no registered products the entry would be retained until the completion of retrospective harmonisation. At this time, the retention or removal of these entries would be considered on their merits.

- Inclusion in Appendix C was not supported as NZ had no equivalent and would still have to retain carbaryl in Schedule 4, and it was debatable whether the toxicity profile warranted inclusion in Appendix C.

- Members supported the retention of the Schedule 4 entry to foster harmonisation with NZ and to maintain existing controls over imports and dispensing by pharmacists.

The outcome of the Committee's consideration was that:

- Members confirmed that the existing scheduling for agricultural and veterinary uses of carbaryl was appropriate on the basis that the toxicity profile was appropriate for inclusion in Schedule 6, and Schedule 5 for preparations containing 10% or less of carbaryl.

- Members did not support the removal of the Schedule 4 entry on the basis that a doctor's prescription should continue to be required for any human therapeutic use of carbaryl.

ACPH

The 20th meeting of the Advisory Committee on Pesticides and Health (19th October 2000) was invited to comment upon OCS's review of the latest data, in particular the following issues:

- The utility of short-term carcinogenicity studies in p53-deficient mice, both with respect to carbaryl and more generally;
- In light of the negative findings in the short-term carcinogenicity study with carbaryl, was there any justification for changing the 4000-fold safety factor upon which the ADI is currently based;
- Whether the Committee agreed with OCS's recommended ARfD for carbaryl;
- What is a toxicologically defensible systemic dose of carbaryl to which persons may be exposed when using carbaryl products within the home;
- Was the Committee in general agreement with the assumptions used in estimating the systemic doses of carbaryl that would be absorbed by persons using or making contact with carbaryl within the home garden/veterinary setting;
- Did the Committee believe that OCS's recommendations with respect to continued registration of HG/HV products are justified;
- Should any further exposure scenarios be considered; and
- Was the human exposure model developed by OCS applicable to other HG/HV pesticides?

Carbaryl was considered again by the ACPH at its 23rd meeting (2nd May 2002). The Committee was advised that since the previous consideration, the following events had taken place:

- CRIH section had reviewed additional toxicology studies on carbaryl, which had strengthened the overall database (particularly in terms of repeat-dose and chronic toxicity in non-rodents, and reproductive toxicity) but not advanced the state of knowledge on the carcinogenicity of carbaryl in rodents and its relevance to humans.
- The JMPR had set an ARfD of 0.2 mg/kg bw for carbaryl, applying a 25-fold safety factor to a NOEL for anticholinesterase effects of 3.8 mg/kg bw/d in a 5-wk dog study.
- The JMPR had reduced the safety factor applied to the 16 mg/kg bw/d LOEL for tumour formation in male mice from 5000-fold to 2000-fold, resulting in an increase in the JMPR ADI for carbaryl from 0.003 to 0.008 mg/kg bw/d. The reduction in the safety factor appeared to have been made in light of the absence of carcinogenic activity in the 6-mo carcinogenicity study with carbaryl in p53 "knockout" mice, together with other supporting evidence that carbaryl is not a genotoxic carcinogen.

OCS requested the ACPH to affirm the proposed Australian ARfD of 0.01 mg carbaryl/kg bw, based on the NOEL for ChE inhibition and behavioural disturbance of 1 mg/kg bw/d in 13-wk and developmental neurotoxicity studies in rats. The request was made on the grounds that there is no reliable NOEL for anticholinesterase effects in humans, and that humans have shown clinical signs of anticholinesterase toxicity at carbaryl doses as low as 2.8 mg/kg bw po, which lies below the canine short-term NOEL for RBC and brain ChE inhibition.

OCS also sought the opinion of the ACPH as to whether there was any justification for the safety factor applied to the pivotal LOEL for tumour formation in male mice to be revised from its current value of 4000.

1.4 DETAILED TOXICOLOGY REPORT

1.4.1 Introduction

Regulatory History of Health Considerations in Australia

A history of the consideration of carbaryl by regulatory committees in Australia since 1990 is detailed below:

Date	Regulatory Activity
February 1990	PACSC: The Committee recommended that the current provisional MRL of 5 mg/kg in raw cereal remain to February 1992, pending production of Australian residue data including oats and barley. The Committee also recommended that the NHMRC MRL Standard be amended to include: Table 3 Carbaryl 100 mg/kg AL 0161 Straw fodder (dry and hay of cereal grains) 100 AL 0161 Cereal grain forage
February 1990	NDPSC: The Committee agreed to clearance of a Rhone Poulenc active constituent based on the fact that the batch analysis was the same as a previously cleared active constituent.
February 1991	PACSC: The Committee requested advice from ACAC as to whether the use of carbaryl in cereals is being progressed and hence whether the MRLs are pertinent.
June 1991	PACSC: On the 5 April 1991 the Committee requested AAVCC to advise whether use in cereals is being progressed.
June 1991	PACSC: The Committee noted an evaluation of additional genotoxicity studies provided by Rhone-Poulenc in support of their active constituent clearance for carbaryl (previously cleared), in which no genotoxicity was observed in in-vitro cytogenetic, forward mutation, UDS and bacterial reversion assays.
August 1991	PACSC: The Committee requires Australia wide registered/approved use patterns to allow consideration of the residue data by a member.
February 1995	ACPH: The Committee examined supplementary toxicology data. Supported proposal to obtain historical control data from other labs and sources re vascular tumours in CD-1 mice.
October 1998	ACPH: The Committee concluded that a clear NOEL had not been demonstrated for vascular tumours in male mice with an LOEL of 16 mg/kg bw/d. The Committee reviewed out of session and agreed to set an ADI of 0.004 mg/kg bw/d on the basis of the LOEL and 4000 fold SF.
February 1999	NDPSC: The Committee recommended the entries for carbaryl be amended to: Schedule 2 Carbaryl –delete entry Schedule 4 Carbaryl for human therapeutic use. Schedule 5 Carbaryl (a) in preparations containing 10 per cent or less of carbaryl **except** when included in Schedule 4; or (b) when impregnated into plastic resin material containing 20 per cent or less of carbaryl. Schedule 6 Carbaryl except when included in Schedule 4 or 5.
April 1999	ACPH: The Committee confirmed out of session decision to set the ADI at 0.004 mg/kg bw/d on the basis of the LOEL of 16 mg/kg bw/d with a safety factor of 4000.

Date	Regulatory Activity
October 2000	ACPH: The Committee reaffirmed the ADI of 0.004 mg/kg bw/d and noted proposed ARfD of 0.01 mg/kg bw based on application of a 100-fold safety factor to a NOEL of 1 mg/kg bw/d in rat 13-wk and developmental neurotoxicity studies. The Committee requested re-consideration of the safety factor in light of the paper by Renwick (2000). However, CRIH section could not find sufficient comparative toxicokinetic or toxicodynamic data to form a basis for reduction of the safety factor.
May 2002	ACPH: Following consideration of additional toxicology studies, the Committee again reaffirmed the ADI of 0.004 mg/kg bw/d and confirmed an ARfD of 0.01 mg/kg bw.
October 2002	NDPSC: The Committee confirmed the existing Poisons Schedule status of carbaryl and did not support a CRIH Section recommendation to remove carbaryl from S4.

Health Standards

NOEL/ADI

In October 1998, the ACPH reconsidered the ADI for carbaryl in light of the expanded toxicological database then available, and the draft NHMRC guidelines for derivation of modifying factors for seriousness of carcinogenic effect. The ACPH recommended that a 4000-fold safety factor be applied to the LOAEL of 100 ppm (16 mg/kg bw/d) for vascular tumours in occurring in male mice in a 2-yr dietary study, giving a revised ADI of 0.004 mg/kg bw/d.

This pivotal study failed to demonstrate a NOEL, as 100 ppm was the lowest feeding level administered. However, several other studies with carbaryl have demonstrated NOELs at lower doses. The lowest NOEL in animal studies is 1 mg/kg bw/d, established in rat subchronic and developmental neurotoxicity studies, based on brain, plasma and erythrocyte ChE depression at the next highest dose 10 mg/kg bw/d.

Poisons Schedule

Carbaryl is classified as a Schedule 6 poison in the SUSDP, with Schedule 5 entries for preparations containing 10 per cent or less of carbaryl, or when impregnated into plastic resin material containing 20 per cent or less of carbaryl. Carbaryl preparations for human therapeutic use are listed in Schedule 4.

International Toxicology Assessments

JMPR

Carbaryl was reviewed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1963, 1965, 1966, 1967, 1969 and 1973. The original ADI of 0-0.02 mg/kg bw/d was set in 1963 on the basis of a NOAEL of 1.8 mg/kg bw/d in a 1-yr dog study. This ADI was confirmed in 1965, 1966 and 1967. In 1969, a temporary ADI of 0-0.01 mg/kg bw/d was established, using an extra safety factor because of concern about effects on the male reproductive system seen in a 1-yr gavage study in rats with a NOAEL of 2 mg/kg bw/d, and because a dose of 0.12 mg/kg bw/d may have affected renal function in a 6-wk study in humans. In 1973, the Meeting established a full ADI of 0-0.01 mg/kg bw/d.

The JMPR carried out a further toxicological review of carbaryl in 1996, and decreased the ADI to 0.003 mg/kg bw/d by application of a 5000-fold safety factor to the LOEL for vascular tumours in male mice.

The JMPR again considered carbaryl in September 2001. The ADI was revised upwards to 0.008 mg/kg bw/d; while the basis for the ADI was unchanged, the safety factor was relaxed to 2000. The JMPR also established an ARfD for carbaryl of 0.2 mg/kg bw, based on a NOAEL for ChE inhibition of 125 ppm (equal to 3.8 mg/kg bw/d) in a 5-wk dietary study in dogs. A safety factor of 25 was applied because ChE inhibition by carbaryl [in rats] is "rapidly reversible and driven by the peak concentration in plasma."

IPCS

The International Programme on Chemical Safety (IPCS) reviewed the toxicology of carbaryl in 1994 (published as Environmental Health Criteria 153). The IPCS review concluded that carbaryl was of low hazard to humans because of its low vapour pressure, rapid degradation, rapid spontaneous recovery of inhibited ChE, and the fact that symptoms usually appear before a dangerous dose has accumulated in the body. However, the review commented upon the (then) lack of modern carcinogenicity studies. Carbaryl residue levels in the food and drinking water were judged to lie far below 0.01 mg/kg bw/d (the then ADI) and were considered unlikely to produce health hazards in the general population. Nevertheless, the report noted the potential for carbaryl overexposure when used for public health purposes in the home or recreation areas if the rules for its application were neglected. The only recommendation that has any bearing on the current Australian Special Review, was that that up to date carcinogenicity studies be performed. In retrospect, the timing of the IPCS review appears to be unfortunate, given that the pivotal modern carcinogenicity studies were completed in 1993, the year before EHC 153 was published, and too late to be incorporated in the IPCS evaluation.

UK MAFF

An evaluation by the British Pesticides Safety Directorate (PSD) was performed in 1995. Assessment of the 1993 carcinogenicity studies led the UK Committee on Carcinogenicity (COC) to conclude that carbaryl should be considered as a potential human carcinogen. Subsequently, the UK MAFF accepted the advice of the Advisory Committee on Pesticides (ACP) to remove home garden and home veterinary carbaryl products from the British market.

US EPA

In October 1996, the US EPA imposed exposure mitigation measures on carbaryl based products. Pending the submission of user exposure studies to the Agency, dusts were removed from uses on trees and ornamental plants higher than chest height, and some applications to pets. The conditions of use of household liquid and dust products were amended to prohibit use more than once per week, and to mandate that gloves be worn during application. Some American registrants voluntarily deleted pet uses rather than amend their product labels; in all, some 34 products were affected.

The US EPA has recently released its Human Health Risk Assessment for carbaryl. The current acute and chronic Reference Doses are both 0.01 mg/kg/d. The ARfD was based on a NOAEL of 1 mg/kg/d in a rat developmental neurotoxicity study, to which an uncertainty factor of 100 was applied. The chronic RfD was derived by applying a 300-fold uncertainty factor to a LOAEL of 3.1 mg/kg/d for inhibition of plasma and brain ChE activity in a chronic

dog study. The former US EPA Reference Dose (RfD) for carbaryl was 0.1 mg/kg bw/d, based on application of a 100-fold uncertainty factor to a NOAEL of 9.6 mg/kg bw/d in a 2-yr rat study, performed in 1961. Carbaryl was categorised as a Class C ("possible human") carcinogen; a linear low dose extrapolation approach was used for risk assessment and a "Q1 unit risk" dose of 8.75 X 10^{-4} mg/kg/d was calculated (US EPA, 2002a,b).

IARC

An IARC evaluation was performed on carbaryl in 1976, which concluded that the then available data did not allow an evaluation of the carcinogenicity of carbaryl in animals to be made. At that time, no case reports or epidemiological studies were available to the Working Group, and the IARC categorises carbaryl as not classifiable as to its carcinogenicity in humans.

1.4.2 Metabolism And Toxicokinetics

Valles B (1999) Carbaryl: Investigation of the metabolism of [^{14}C]-carbaryl following 14 days administration to the male CD1 mouse Study No. SA 97481 Lab: Rhone-Poulenc Agro, Centre de Recherche, rue Dostoievski, Sophia Antipolis, France Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC USA Study duration: July 3 to November 20, 1998 Report date: June 16, 1999

QA: yes. GLP: OECD (1982), EC (1986), US EPA (1989) and France (1990) Test Guideline: none cited.

Study design:

A metabolism study was conducted in male CD1 mice (Charles River France, initially aged 4 wk and weighing 17.4 – 25.4 g) following 14 d administration of carbaryl in the diet (Rodent diet AO4C, UAR France) at 0, 10, 100, 1000 or 8000 ppm (9 or 10 animals/group). The test chemical was obtained from Rhone-Poulenc, Research Triangle Park, NC USA (batch 208115110, purity 99%). The pre-treatment phase was followed by a single 50 mg/kg (200 μCi/kg) oral dose of [1-^{14}C]-carbaryl (same source as unlabelled carbaryl, batch CSL-92-360-5-31, radiopurity ≥98%, specific activity 22.04 mCi/mmol, gavaged at a rate of 0.25 g/25 g bw).

During a 10 d acclimation period and dietary administration, animals were housed individually in suspended stainless steel mesh cages within a controlled and monitored environment. Food and filtered, softened tap water were freely available. Food consumption was recorded weekly during the dietary treatment phase, and bodyweights were measured on d –1, 1, 8 and 14, and again immediately prior to gavage dosing. Thereafter, mice were transferred to glass metabolism cages. Urine, faeces and cage washings were collected at 24 h intervals until 168 h post-gavage, when the animals were exsanguinated under Imalgene 500 anaesthesia.

Radioactivity was measured in urine, cage washings, blood, tissues and faeces by LSC. Prior to counting, faeces samples were combusted and the resultant CO_2 was trapped, while tissues were solubilised in alcoholic 2M KOH. HPLC was used to quantify metabolites in pooled urine samples. Aliquots of d 1 urine from 8000 ppm mice were subjected to beta-

glucuronidase or sulfatase hydrolysis before HPLC analysis. Carbaryl metabolites were identified by comparison with certified standards and LC/MS.

Results:

Homogeneity of the dietary mixtures was verified by HPLC at preparation, and the true carbaryl concentrations lay between 100 and 112% of their nominal values. Stability of the 10 ppm mixture was found to be at least 90% after1 wk at room temperature, and after 1 wk frozen storage followed by 1 wk at room temperature. Although individual animal data on food consumption were presented, test compound consumption was not calculated. Mean carbaryl intake at 10, 100, 1000 and 8000 ppm would have been equivalent to approximately 1.5, 15, 150 and 1200 mg/kg bw/d.

Total recovery of radioactivity ranged from 89 to 101% among the various groups. Pre-treatment doses of carbaryl did not influence the absolute amount of radioactivity excreted, some 53 – 67% of which appeared in the urine within 48 h post-gavage. If cage wash radioactivity is assumed to be of urinary origin, approximately 80% of the administered dose was excreted via the kidneys over the entire 168 h. Between 11 and 18% of the radioactivity was excreted via the faeces over the first 48 h, increasing only to 12 – 19% by study termination. The proportion of the administered radioactivity that appeared in faeces tended to rise with increasing pre-treatment dose (see Table). Radioactivity in blood and residual carcass accounted for only <0.5% and <0.2% of the gavage dose, respectively, at 168 h post-gavage.

Up to 21 radioactive components were detected in the urine, in which the major components were dihydrodihydroxy-naphthyl sulfate, alpha-naphthyl sulfate, alpha-naphthyl glucuronide and a co-eluting mixture of hydroxycarbaryl glucuronide and dihydrodihydroxy carbaryl (see Table). Unchanged carbaryl was not detected in the urine. Faecal metabolites were not characterised.

Pretreatment at 8000 ppm elicited a change in the urinary metabolite pattern, seen as a doubling in the proportion of dihydrodihydroxy-naphthyl sulfate and a more modest increase in hydroxy-carbaryl glucuronide/dihydrodihydroxy carbaryl (see Figure). Rhone-Poulenc hypothesise that these metabolites are formed via epoxide intermediates. Based on the information provided, the 8000 ppm group metabolised approximately 25% of the radiolabelled carbaryl dose via epoxidation over 96 h, compared with 17% by the control group. There was no enhancement of epoxidation at 1000 ppm or below. Also at the 8000 ppm pretreatment level, was a concomitant decline in the urinary excretion of some minor unidentified metabolites, which were considered by the study authors to have been formed by alkyl oxidation (see Table and Figure).

The influence of dietary pretreatment on hydrolytic activity against carbaryl, was enigmatic. Compared with controls, there was a slight decline in excretion of the major hydrolysis product alpha-naphthyl sulfate, that was more marked among the 10 and 100 ppm groups than at the two higher doses. Production of alpha-naphthyl glucuronide, the remaining major hydrolysis product, appeared to be inhibited by pretreatment at 10 and 100 ppm, but was slightly enhanced at 1000 and 8000 ppm (see Table). Overall, about 30% of the radiolabelled carbaryl was hydrolysed after no pretreatment or dietary pretreatment at 1000 and 8000 ppm, whereas a corresponding value of approximately 20% was obtained in the 10 and 100 ppm groups.

Excretion of Radioactivity from Male Mice over 96 h Following Gavage with 50 mg/kg [^{14}C]-Carbaryl (% Administered Radioactivity Equivalents)

Pretreatment carbaryl dose, ppm in diet	0	10	100	1000	8000
Urinary Metabolite 1*	3.2	3.0	3.7	3.8	6.8
Urinary Metabolite 12**	14.2	11.8	14.9	13.7	18.7
Urinary Metabolite 16#	14.1	10.9	10.8	12.0	12.0
Urinary Metabolite 18##	16.7	10.8	12.8	19.5	19.5
Combined minor urinary metabolites	19.8	18.8	21.9	19.6	12.0
Total urinary radioactivity	68.0	55.3	64.1	68.6	69.0
Total faecal radioactivity	12.1	15.3	18.3	16.3	18.6

* dihydrodihydroxy naphthyl sulfate (generated via epoxidation)
** hydroxycarbaryl glucuronide and dihydrodihydroxy carbaryl (generated via epoxidation)
# alpha-naphthyl sulfate (generated via hydrolysis)
alpha-naphthyl glucuronide (generated via hydrolysis)

Conclusions:

The study authors concluded that when administered to male mice in the diet at 8000 ppm, carbaryl induces its own metabolism via epoxidation, as evidenced by the increased urinary excretion of dihydrodihydroxy naphthyl sulfate and hydroxycarbaryl glucuronide/dihydrodihydroxy carbaryl.

Comment:

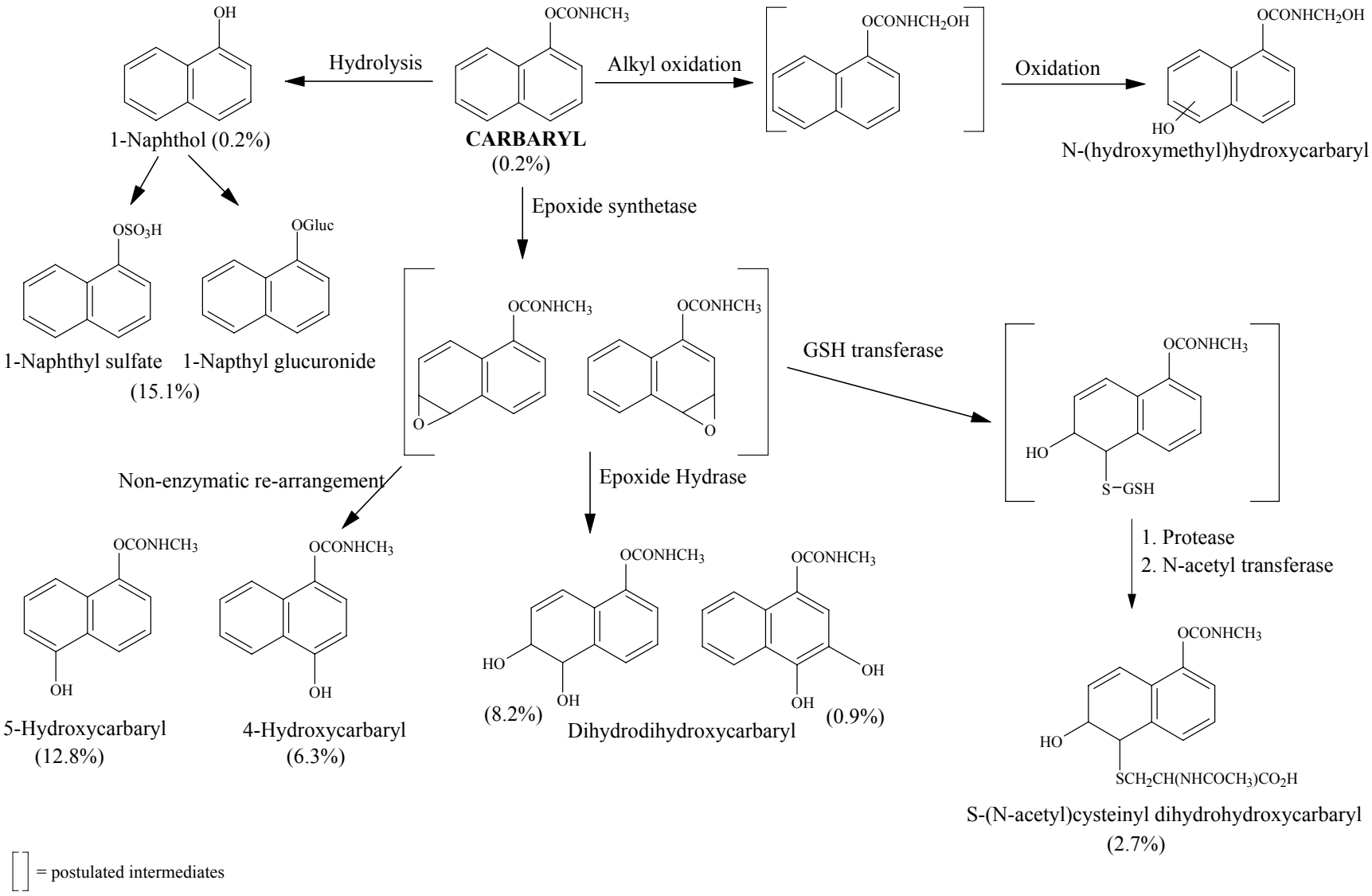
In a previously evaluated study in CD (SD-derived) rats (Totis, 1997), metabolism of carbaryl also proceeded mainly via epoxidation and hydrolysis.

Epoxidation gave rise principally to dihydrodihydroxy carbaryl (Metabolite 12 above) and a glucuronide of that metabolite (not detected in mouse urine). However, the dihydrodihydroxy naphthyl sulfate produced via epoxidation by mice, was not detected in rats. Epoxidation accounted for approximately 20% of the urinary metabolites in rats that had not received dietary pre-treatment with carbaryl. The hydrolysis pathway generated the same metabolites (16 and 18) as in the mouse, and was about twice as active as the epoxidation pathway.

Dietary pretreatment with 7500 ppm carbaryl caused an approximate doubling in the urinary excretion of metabolite 12, raising the proportion of epoxidised carbaryl metabolites to at least 30%. Conversely, production of hydrolysed metabolites declined from 40 to no more than 30% of total urinary metabolites. A similar although slighter trend was evident at 1500 ppm, but dietary pretreatment with 250 ppm carbaryl had no influence on its subsequent metabolism.

Thus, notwithstanding the differences in the epoxidised metabolites produced by mice and rats, and the smaller response in mice, a similar tendency towards increased carbaryl epoxidation at high doses was evident in the two species.

A generalised metabolic pathway for carbaryl in rats is shown on the following page (the study report did not contain a pathway diagram for mice). Figures in parentheses refer to the amount of the metabolites detected as a percentage of administered radioactivity.


OH
1-Naphthol (0.2%)
Hydrolysis
OCONHCH3
CARBARYL
(0.2%)
Alkyl oxidation
OCONHCH2OH
Oxidation
OCONHCH2OH
HO
N-(hydroxymethyl)hydroxycarbaryl
OSO3H
1-Naphthyl sulfate
(15.1%)
OGluc
1-Napthyl glucuronide
Epoxide synthetase
OCONHCH3
OCONHCH3
O
O
GSH transferase
OCONHCH3
HO
S−GSH
1. Protease
2. N-acetyl transferase
Non-enzymatic re-arrangement
Epoxide Hydrase
OCONHCH3
OH
5-Hydroxycarbaryl
(12.8%)
OCONHCH3
OH
4-Hydroxycarbaryl
(6.3%)
OCONHCH3
HO
OH
(8.2%)
OCONHCH3
OH
OH
(0.9%)
Dihydrodihydroxycarbaryl
OCONHCH3
HO
SCH2CH(NHCOCH3)CO2H
S-(N-acetyl)cysteinyl dihydrohydroxycarbaryl
(2.7%)
[ ] = postulated intermediates

1.4.3 Short-Term Repeat-Dose Study

Dogs

Hamada NN (1991) Subchronic toxicity study in dogs with carbaryl technical Study No. 656-152 Lab: Hazleton Laboratories America, Vienna, VA, USA Sponsor: Rhone-Poulenc Ag Co, Research Triangle Park, NC USA Study duration: November 21 – December 29, 1989 Report date: March 28, 1991

QA: Yes GLP: US EPA (40 CFR Part 160) Test guideline: US EPA Subdivision F, Series 82-1

Study design

Carbaryl technical (lot 87191, 99.3% pure, from Rhone-Poulenc) was administered to beagle dogs (initially aged approximately 6 mo and weighing 6.4-9.1 kg [M] or 5.8-8.3 kg [F], from Hazleton Research Products, Cumberland, VA, USA) in the diet at concentrations of 0, 20, 45 or 125 ppm for approximately 5 wk. There were 6 dogs/sex/feeding level.

The test chemical was blended with approximately 200 g diet (Purina Certified Canine Diet meal #5007) to form pre-mixes, which were then added to and blended with the required amount of feed. Separate mixtures were prepared for each carbaryl concentration. Mixed diets were prepared weekly and stored frozen for about 1 wk prior to use. Duplicate samples were withdrawn from each preparation. One of each pair of samples was used for analysis of carbaryl concentration, which was performed weekly. The other sample was stored frozen. The 20 and 125 ppm diets (and one sample of the control diet) were also analysed for homogeneity. Stability analyses were carried out on 20 ppm diet on the day of preparation, after 7 d frozen storage, and after 7 d frozen storage followed by a further 6 or 11 d at room temperature.

Dogs were permitted a 3-wk acclimatisation period before the start of treatment. They were housed individually in stainless steel cages within a controlled and monitored laboratory environment. Approximately 2.5 kg feed/wk was offered to each dog. Feed was provided for 2 h/d but there was free access to water. Animals were observed twice daily for mortality and moribundity, and at least once daily for clinical signs. Cageside observations were performed approximately 3 h after withdrawal of feed. Body weights and feed consumption were recorded weekly. Ophthalmoscopic examinations were made on all dogs pre-treatment and during wk 5, using tropicamide (1% Mydriacyl) as a mydriatic.

Plasma and RBC ChE assays were performed on each dog 11, 8 and 5 d before treatment and on study days 14 and 32. Brain ChE activity was measured at termination on d 37-39. Sample collection was initiated approximately 2 h after the feeding period had ended. ChE activity in plasma, RBC and brain homogenates was assayed in an autoanalyser using acetylthiocholine as the substrate.

At termination, dogs were exsanguinated under sodium thiamylal anaesthesia. Necropsy included examination of the external surface of the body, all orifices, cranial cavity, carcass, external and cut surfaces of the spinal cord, nasal cavity and paranasal sinuses, thoracic, abdominal and pelvic cavities and their viscera, and cervical tissues and organs. Lesions were preserved in 10% formalin for possible future examination.
Body weight gain, cumulative food consumption and ChE activity data from the control group were compared statistically to data from the treated groups of the same sex. All data appear to

have been tested by Levene's test of homogeneity of variances, with heterogeneous data being transformed by Log_{10}, square, square root, reciprocal, arcsine or rank transformation to obtain homogeneity. All data were then subjected to ANOVA. Tests for homogeneity of variance and ANOVA were evaluated at the 5% 1-tailed probability level. Dunnett's test was used for comparison of control and test group data if ANOVA was significant. Control *vs*. treatment group mean comparisons were evaluated at the 5% 2-tailed probability level.

Results

The dietary preparations were homogeneous, with carbaryl concentrations of 92-102% of target values. Stability of carbaryl in the 20 ppm diet mix was adequate, with the assayed concentration being 94% of the target value after 7 d frozen storage, and 91% of the target value after 7 d frozen storage followed by 11 d at room temperature. Weekly carbaryl concentrations were 90-102% of target values throughout the study.

No dogs died prematurely, and there were no treatment-related clinical signs or effects on bw gain, food consumption or ophthalmological findings. Mean achieved carbaryl intakes across the study were 0.59, 1.4 and 3.8 mg/kg bw/d for males and 0.64, 1.5 and 4.1 mg/kg bw/d for females at the 20, 45 and 125 ppm feeding levels, respectively.

Mean plasma ChE activity in the treated male groups was lower than among controls throughout the study, including the baseline measurements. Statistical significance was achieved at 20 and 125 ppm on d 14, when these two groups showed a 16% and 23% deficit, respectively, against the control mean (see table below). However, statistical significance was not attained at the final measurement on d 32, due mainly to a decline in ChE activity among controls. When compared with the pre-treatment value on d –5, ChE activity in the 1500 ppm male group was 20-21% lower after 14 and 32 d of treatment, which is considered by the reviewing toxicologist as probably biologically significant. By contrast, the decline in plasma ChE activity at 20 and 45 ppm was less than 15% compared with the baseline value, and is not considered to be a treatment-related effect.

Interpretation of treatment-related effects in females is made difficult by the fact that the female control group had the lowest pre-study ChE activity, and was consistently lower than the 20 and 45 ppm female groups. Pairwise comparisons against the controls were statistically non-significant at all doses. When compared with their baseline value on d –5, however, the 1500 ppm females showed a 23% decline in ChE activity.

Taken together, these findings suggest there was treatment-related inhibition of plasma ChE activity at 1500 ppm, which lay outside the statistical threshold of detection because of confounding by the small number of dogs per group and a time-related downward trend in control ChE activity.

There were no treatment-related effects on RBC or brain ChE activity, or on gross necropsy findings.

Plasma ChE activity (μmol/mL, mean ± SD) in dogs (n=6/group) during study 656-152
Percent inhibition against baseline (day –5) ChE activity is shown in brackets

Carbaryl ppm	Sex	Study day –5	Study day 14	Study day 32
0	**M**	9.5 ± 0.6	8.9 ± 0.6 (6)	8.3 ± 0.8 (13)
20	**M**	8.1 ± 1.3	7.3* ± 1.3 (10)	7.1 ± 1.1 (12)
45	**M**	8.9 ± 1.2	8.1 ± 1.1 (9)	7.6 ± 0.8 (15)
125	**M**	8.7 ± 2.1	6.9* ± 1.2 (21)	7.0 ± 1.7 (20)
0	**F**	7.9 ± 0.7	7.9 ± 0.9 (0)	7.4 ± 0.7 (6)
20	**F**	8.9 ± 1.5	8.8 ± 1.4 (1)	8.0 ± 1.1 (10)
45	**F**	8.8 ± 1.0	8.3 ± 1.2 (6)	8.0 ± 1.1 (9)
125	**F**	9.2 ± 0.9	7.8 ± 0.6 (15)	7.1 ± 0.7 (23)

*significantly different from control, $p<0.05$

Conclusions

It is impossible to exclude treatment-related depression of plasma ChE activity at the highest dose of 125 ppm, given that up to a 23% decrease in ChE activity occurred relative to pre-treatment activity. Consequently, the NOEL is set at 45 ppm (equal to 1.4 mg/kg bw/d).

Comment

In the following 1-yr study with carbaryl in dogs at the same laboratory, there was unequivocal depression of plasma ChE activity at 125 ppm after 5 weeks' treatment, and also at 13 wk.

1.4.4 Chronic Toxicity Study

Dogs

Hamada NN (1987) One-year oral toxicity study in beagle dogs with carbaryl technical Study No. 400-715 Lab: Hazleton Laboratories America, Vienna, VA, USA Sponsor: Union Carbide Corp, Research Triangle Park, NC USA Study duration: July 16, 1985 – July 18, 1986 Report date: March 18, 1987

QA: Yes GLP: US EPA (40 CFR Part 160) Test guidelines: US EPA Subdivision F, Series 83-1, OECD 453 (May 1981)

Study design

Carbaryl technical (lot 17909 A-6, 99% pure, from Union Carbide Agricultural Products Co, Inc) was administered to beagle dogs (initially aged 20-21 wk and weighing 6.0-8.3 kg [M] or 5.5-7.2 kg [F], from Hazleton Research Products, Denver, PENN, USA) in the diet at concentrations of 0, 125, 400 or 1250 ppm for 12 mo. There were 6 dogs/sex/feeding level.

The feeding levels were based on the results of a 2-week rangefinding study, in which groups of 1 dog/sex received 50, 100, 200, 400, 800 or 1600 ppm carbaryl by dietary admixture for 14 d. There were no controls. All dogs survived. Reaction to treatment was confined to salivation,

which occurred in the 1600 ppm female. Plasma and RBC ChE activity was inhibited by >25% (compared with pretreatment values) at and above 200 ppm. Inhibition was maximal from 2-4 h post-feeding and did not appear to reverse within 6 h. There was a dose-related downward trend in brain ChE activity in females but not males, which is difficult to interpret in the absence of control data. No other treatment-related effects were reported.

In the main study, carbaryl was blended with approximately 200 g diet (Purina Certified Canine Diet meal #5007) to form pre-mixes, which were then added to and blended with the required amount of feed. Separate mixtures were prepared for each carbaryl concentration. Mixed diets were prepared weekly and stored frozen until use. Samples of control and test diet preparations were retained, and samples from wk 1-4 and every 4th wk thereafter were used for analysis of carbaryl concentration. The test diets were also analysed for homogeneity prior to study commencement. Stability analyses were carried out on frozen diets and diets held at room temperature for 0, 3, 7, 14 and 21 d after preparation. The assayed samples contained 50, 400 and 1600 ppm carbaryl and had been prepared for the dose-ranging study.

Dogs were permitted a 27-d acclimatisation period before the start of treatment. They were housed individually in stainless steel cages within a controlled and monitored laboratory environment. Feed was provided for 2 h/d but there was free access to water. Animals were observed twice daily for mortality and moribundity, and at least once daily for clinical signs. Body weights and feed consumption were recorded weekly. Ophthalmoscopic examinations were made on all dogs pre-treatment and during wk 52 using tropicamide (1% Mydriacyl) as a mydriatic.

Plasma and RBC ChE assays were performed on each dog 3, 2 and 1 wk before treatment and on study wk 5, 13, 26 and 52. Brain ChE activity was measured at study termination. Sample collection was initiated approximately 2 h after the feeding period had ended. ChE activity in plasma, RBC and brain homogenates was assayed in an autoanalyser. To further characterise the cholinesterase inhibitory effects of carbaryl, I_{50} concentrations of the test chemical in plasma and RBC were determined *in vitro* using blood obtained from untreated beagle dogs.

Two weeks before study commencement and during wk 13, 26 and 52, the following haematological parameters were measured: haemoglobin concentration, haematocrit, erythrocyte count, total leukocyte count, leukocyte differential count, platelet count, clotting time, prothrombin time, Heinz bodies, and cell morphology. At these same time points, the following clinical chemistry assays were performed: albumin, globulin, albumin:globulin ratio, total protein, sodium, potassium, calcium, chloride, inorganic phosphorus, glucose, total cholesterol, AST, GGT, ALP, LDH, CPK, ALT, creatinine, methaemoglobin, BUN, direct bilirubin and total bilirubin. Urinalysis (colour/appearance, overnight volume, specific gravity, pH, occult blood, protein, glucose, urobilinogen, bilirubin, ketones, sediment and reducing substances) were also carried out at wk 13, 26 and 52.

Routine clinical pathology samples were collected in the morning before the daily feeding period and on the day following collection of blood for ChE assay. Urine samples were from cage pan runoff. The dogs were water fasted during urine collection.

At termination, dogs were exsanguinated under sodium thiamylal anaesthesia. Necropsy included examination of the external surface of the body, all orifices, cranial cavity, carcass, external and cut surfaces of the brain and spinal cord, nasal cavity and paranasal sinuses, thoracic, abdominal and pelvic cavities and their viscera, and cervical tissues and organs.

Terminal bw and the following organ weights were recorded: adrenals, brain, gonads, heart, kidneys, liver, spleen, thyroid with parathyroid and pituitary. Gross lesions and the following tissues were preserved in 10% formalin and examined histologically: brain, spinal cord, eyes, pituitary, salivary glands, heart, thoracic aorta, thymus, thyroid, lungs, trachea, spleen, sternum, lymph nodes, sciatic nerve, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, adrenal glands, pancreas, liver with gallbladder, kidneys, urinary bladder, kidneys, urinary bladder, gonads, prostate, epididymis, vagina, uterus/cervix, skin, mammary gland and thigh muscle.

Body weight, weekly and cumulative food consumption, clinical pathology (excluding cell morphology and urinalysis data) and organ weight data from the control group were compared statistically to data from the treated groups of the same sex. All data appear to have been tested by Levene's test of homogeneity of variances, with heterogeneous data being transformed by Log_{10}, square, square root, reciprocal, arcsine or rank transformation to obtain homogeneity. All data were then subjected to ANOVA. Tests for homogeneity of variance and ANOVA were evaluated at the 5% 1-tailed probability level. Dunnett's test was used for comparison of control and test group data if ANOVA was significant. Control *vs*. treatment group mean comparisons were evaluated at the 5% 2-tailed probability level.

Analysis of RBC and plasma ChE data was initially performed on the basis of a factorial design with repeated measures using the P2V program of BMOP (U Cal, 1983). The 3 pretreatment values for each dog were averaged, and the mean was used as a covariate. Since there were no significant differences among the groups with respect to the covariates, each group was then analysed by single factor repeated measures ANOVA and Dunnett's t-test (unadjusted) to isolate any within-group time effect *vs* pretreatment or baseline value. Treatment-group comparisons were also performed with all groups by single factor ANOVA and Dunnett's t-test procedures. Brain ChE activity was evaluated by single factor ANOVA and Dunnett's t-test. The two sexes were evaluated separately.

Results

Stability analysis showed that 92-96% of initial carbaryl levels were maintained over 3 wk frozen storage in the experimental diet. The diets were homogeneous and assayed carbaryl concentrations lay between 97 and 111% of their target values. Achieved carbaryl doses were presented for each study wk and exhibited a gradual decrease from their initial values. A table comparing the initial, final and approximate overall mean doses appears below:

Achieved mean carbaryl doses (mg/kg bw/d) in dogs, study 400-715

Group	Mean over wk 1-5	Mean over wk 48-52	Approximate mean over wk 1-52
125 ppm M	3.9	3.0	3.5
125 ppm F	3.9	3.4	3.8
400 ppm M	12	10	11
400 ppm F	12	9.7	11
1250 ppm M	36	31	34
1250 ppm F	34	33	34

No dogs died during the study and there were no treatment-related clinical signs. Bodyweight gain was inhibited to a biologically significant extent in both sexes at 1250 ppm during the first 5 wk of treatment. The 1250 ppm female group gained slightly less weight than controls throughout the study (see table below) but the effect did not persist in the males. Reduced bw gain in the 1250 ppm females was accompanied by reduced feed consumption, particularly between wk 1 and 5. However, there were never any statistically significant effects on group mean bw, and terminal bw was closely comparable among the various treated and control groups.

In haematology, leucocyte and segmented neutrophil counts became statistically and biologically significantly elevated in 1250 ppm males (see table). Significantly ($p<0.05$) shortened clotting time in 400 and 1250 ppm males at wk 26 is considered to be an artefact arising from a marked increase in the control value.

Carbaryl caused inhibition of ChE activity at all 3 feeding levels. The effects were clearly dose-related, were more marked in females, and (in the case of plasma and RBC ChE) were greater at wk 5 and 13 than subsequently. The time-related trend towards decreasing inhibition may arise from the gradual decline in delivered carbaryl dose that was observed in treated groups as the study progressed.

Plasma ChE inhibition *vs* control was 47-66% at 1250 ppm ($p<0.05$ throughout the study), was 9-36% at 400 ppm ($p<0.05$ throughout the study in males and at 5, 13 and 26 wk in females), and was 12-23% in 125 ppm females ($p<0.05$ at 5, 13 and 26 wk) (see table). When compared against the mean of the 3 pretreatment values, plasma ChE inhibition was 53-64% at 1250 ppm and 21-37% at 400 ppm, but 15% or less at 125 ppm. *In vitro*, plasma ChE activity was inhibited by 50% at approximately 10^{-5} M carbaryl.

RBC ChE inhibition *vs* control was 30-56% at 1250 ppm ($p<0.05$ throughout the study) and 19-34% at 400 ppm ($p<0.05$ at 5, 13 and 26 wk in females but only at 5 and 13 wk in males) (see table). When compared against the mean of the 3 pretreatment values, RBC ChE inhibition was 26-45% at 1250 ppm and 8-33% at 400 ppm, but 17% or less at 125 ppm. Carbaryl inhibited RBC ChE activity *in vitro* by 50% at a concentration of approximately 10^{-6} M.

Brain ChE activity was depressed by 14-32% in the treated male groups but failed to attain statistical significance against control, while treated females showed significant ($p<0.05$) inhibition at all doses (see table).

In clinical chemistry, the female 1250 ppm group had slight but significant ($p<0.05$) depression in albumin concentration at all measured time points, together with increased inorganic phosphorus at wk 52 (see table). Although significant ($p<0.05$) depressions in ALT and AST occurred in the treated male groups at wk 52, these were devoid of biological significance, having arisen from increases in the mean control values. Mean CPK activity fluctuated markedly in the treated groups throughout the study, sometimes being elevated by 8-fold or at other times being depressed by 50% compared with control means. There was never any dose-response relationship and the direction of the departure from control was inconsistent between the sexes at study termination. Individual animal data showed that group mean elevations were attributable to gross increases in 1 or 2 individuals, rather than being consistent within the group. The findings are therefore not considered to be suggestive of skeletal muscle toxicity, but may indicate muscular trauma caused by poor venipuncture technique.

There were no treatment-related urinalysis or ophthalmological findings. Absolute and relative liver weights were increased in 1250 ppm males (see table), which was considered biologically significant by the study authors. Relative thyroid weight was decreased ($p<0.05$) in this same group, but was not considered biologically significant in the absence of a treatment-related effect on absolute thyroid weight. There were no treatment-related gross or histopathological findings.

Statistically and biologically significant effects in dogs, study 400-715 (data presented as mean ± SD or as incidence, where appropriate)

Parameter	Carbaryl concentration in diet (ppm)							
	Males				1.4.4.1.1 Females			
	0	125	400	1250	0	125	400	1250
No. animals	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
Bw gain, wk 0-5 (kg)	1.1 ± 0.3	1.3 ± 0.4	1.2 ± 0.6	0.7 ± 0.5	1.0 ± 0.2	1.1 ± 0.3	1.0 ± 0.3	0.5* ± 0.2
Bw gain, wk 0-52 (kg)	2.9 ± 0.8	3.3 ± 1.1	3.2 ± 1.7	3.5 ± 1.6	2.4 ± 0.5	2.7 ± 0.8	2.3 ± 0.7	2.1 ± 0.5
Cumulative feed consumption, wk 1-5 (g)	7831 ± 1076	8661 ± 1173	8501 ± 833	7818 ± 1371	7409 ± 373	7547 ± 626	7745 ± 568	6709 ± 646
Terminal bw (kg)	9.6 ± 1.4	10.4 ± 1.5	10.2 ± 1.7	10.8 ± 1.6	8.7 ± 0.6	8.9 ± 1.0	8.8 ± 0.8	8.8 ± 0.8
Leucocyte count, wk 52 (x1000/µL)	10.3 ± 2.0	11.1 ± 2.1	9.9 ± 1.5	15.2* ± 3.2	8.5 ± 1.2	9.3 ± 1.4	9.7 ± 2.2	10.9 ± 1.6
Segmented neutrophil count, wk 52 (x1000/µL)	7.0 ± 1.8	8.2 ± 2.0	7.5 ± 1.8	11.4* ± 2.5	5.3 ± 1.1	6.4 ± 1.1	6.7 ± 1.4	7.3 ± 1.6
Plasma ChE[#] activity, wk 13 (µmol/mL)	8.6 ± 1.9	7.5 ± 1.2	5.7* ± 1.1	3.7* ± 1.0	8.6 ± 0.9	6.6* ± 0.8	6.2* ± 1.2	3.7* ± 0.7
RBC ChE[#] activity, wk 13 (µmol/mL)	7.2 ± 1.4	6.2 ± 1.5	5.2* ± 0.6	3.7* ± 0.8	8.6 ± 1.9	8.3 ± 1.7	6.1* ± 0.7	6.1* ± 0.8
Brain ChE activity, wk 52 (µmol/mL)	11.3 ± 3.4	9.7 ± 2.9	7.7 ± 2.1	8.5 ± 1.4	9.0 ± 1.2	7.2* ± 0.6	7.0* ± 1.2	5.8* ± 0.5
Albumin conc., wk 52 (g/dL)	3.3 ± 0.2	3.3 ± 0.2	3.2 ± 0.1	3.2 ± 0.2	3.5 ± 0.2	3.3 ± 0.3	3.4 ± 0.1	3.1* ± 0.2
Inorganic P conc., wk 52 (mg/dL)	3.0± 0.5	3.6 ± 0.6	3.1 ± 0.6	3.8 ± 0.3	3.2 ± 0.3	3.2 ± 0.4	3.5 ± 0.5	4.0* ± 0.7
Absolute liver weight (g)	242 ± 37	255 ± 18	269 ± 36	301* ± 25	247 ± 18	247 ± 49	234 ± 31	259 ± 35
Liver weight relative to bw (%)	2.6 ± 0.3	2.5 ± 0.3	2.7 ± 0.3	2.8 ± 0.6	2.9 ± 0.3	2.8 ± 0.4	2.6 ± 0.3	3.0 ± 0.2

[#]Wk 13 data shown because effects at wk 5 and 13 were similar and more severe than at wk 26 and 52
*$p<0.05$ *vs* control

Conclusions

Based on statistically significant depression of plasma and brain ChE activity in female dogs treated at the lowest dose of 125 ppm (approximately 3.8 mg/kg bw/d), the study is considered not to have demonstrated a NOEL. The study authors did not consider the anticholinesterase effects of carbaryl at the lowest dose to be biologically significant, and set the NOEL at 125 ppm.

1.4.5 Carcinogenicity Studies

Mice

Chuzel F (1999) Carbaryl 6-month carcinogenicity study in p53 knockout mice by dietary administration Study No. SA 98155 Lab: Rhone-Poulenc Agro, Centre de Recherche, rue Dostoievski, Sophia Antipolis, France Sponsor: Rhone-Poulenc Agro, rue Pierre Baizet, Lyon, France Study duration: July 20, 1998 to May 6, 1999 Report date: July 08, 1999

QA: yes. GLP: OECD (1997), EC (1986), France (1990), US EPA (1989), Japan (1984). No test guidelines apply to this special protocol.

Study design:

A subchronic carcinogenicity study was performed with carbaryl in "knockout" mice, heterozygous for the p53 tumour suppressor gene. The mouse strain used (C57Bl/6 Tac fBR-[KO]Trp53N5-T, obtained from Taconic, Germantown, NY USA via Bomholtgard Breeding and Research Center AIS, Denmark) is phenotypically normal, but has enhanced susceptibility to genotoxic events.

Carbaryl (Rhone-Poulenc, Institute Plant, WV USA, batch 208115110, purity 99%) was administered via the diet to groups of 20 males at concentrations of 10, 30, 100, 300, 1000 or 4000 ppm for at least 180 d. A further group of 20 control males received plain diet. At commencement of treatment, the mice were 9 – 11 wk old and weighed 22 – 29 g.

The test chemical was incorporated into the powder diet (M 20 controle, supplied by Pietrement, Provins, France) by dry mixing. Stability of 10 and 4000 ppm carbaryl in the diet was investigated prior to the study, under conditions of frozen storage for 3, 6 or 14 wk followed by 1 wk at ambient temperature. Carbaryl was stable under these conditions at 4000 ppm, but the 10 ppm mixture was not stable beyond 6 wk and therefore fresh mixtures were prepared at 5- or 6-weekly intervals during the study. The concentration of carbaryl in each batch of the test diets was verified by HPLC.

Animals were housed individually in suspended stainless steel wire mesh cages under standard conditions in a controlled and monitored laboratory environment. Feed and water were available *ad libitum* except for overnight fasting before sacrifice. Mice were checked for mortality and moribundity twice daily on weekdays and daily on weekends. Clinical signs were recorded daily, while a detailed physical examination was performed weekly. Bodyweights and food consumption were measured weekly during the first 14 wk and at monthly intervals thereafter.

On study d 181-184, surviving mice were exsanguinated under pentobarbital anaesthesia (60 mg/kg IP). All animals were necropsied, whether dying intercurrently or sacrificed terminally. Brain, heart, kidneys, liver, spleen, testes and thymus were weighed (at scheduled sacrifice only). In addition to macroscopic abnormalities, the following organs/tissues were fixed and embedded: adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), brain, epididymis, oesophagus, eye and optic nerve, gall bladder, Harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx, liver*, lung, lymph nodes (submaxillary, mesenteric), pancreas, pituitary gland, prostate, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen*, stomach, salivary gland, testis, thymus*, thyroid (with parathyroid), tongue, trachea, and urinary bladder*. All tissues from all control and 4000 ppm mice and all decedents, were examined microscopically. Macroscopic lesions and tissues marked with an asterisk were examined in the intermediate dose groups.

Bodyweight, bodyweight change and food consumption were compared between exposed groups and controls by Bartlett's test for homogeneity of variances. If insignificant, analysis of variance (ANOVA) was performed. When variance was significant, Dunnett's test was performed to compare group and control means. If Bartlett's test indicated heterogeneous variances, it was followed by the Kruskal-Wallis non-parametric 1-way analysis of variance by ranks. If the Kruskal-Wallis test was significant, Mann-Whitney's test was used to compare each group to the control group. Statistical analyses were performed using the SAS program.

Using the Pathtox computer system, organ weights, terminal bodyweights, organ:brain and organ:body weight ratios (except for testis) were compared by Dunnett's test when Bartlett's test indicated homogeneous variances. If variances were non-homogeneous, variables were analysed using SAS programs. Kruskal-Wallis non-parametric 1-way analysis of variance was run and, if significant, was followed by the Mann-Whitney test to compare each treated group to the control group. Statistical analysis of testis weight was performed with the SAS program only.

Results:

Concentrations of the test compound in the diet lay between +10 and –12% of nominal values. Mean achieved doses throughout the study were 1.8, 5.2, 17.5, 52, 165 and 717 mg/kg bw/d at 10, 30, 100, 300, 1000 and 4000 ppm, respectively.

Carbaryl did not induce mortality or clinical signs. Intercurrent deaths or humane sacrifices were limited to 1 at 10 ppm and 2 at each of 30 and 300 ppm. Treatment-related observations were confined to the 4000 ppm group, which displayed a slight but significant ($p<0.01$ *vs.* control) deficit in food consumption during wks 2 – 5 and 7-8, correlated with lower mean bodyweight ($p<0.05$ or 0.01 *vs.* control at wks 6, 7, 10 and 11). Bodyweight change did not show any consistent effect of treatment on a week by week basis, but at study termination the 4000 ppm group mean bodyweight remained approximately 8% below the control value (see Table). A transient decrease in food consumption among the 1000 ppm group ($p<0.05$ *vs.* control, wks 7-8) was not accompanied by decreased growth or bodyweight.

At termination, an increase was noted in absolute and relative (to body and brain) liver and kidney weights at 1000 and 4000 ppm, while depression in thymus weight (absolute and relative to body and brain weight) occurred at 4000 ppm only (see Table). Statistical significance ($p<0.05$ or 0.01 *vs.* control) was attained with respect to most but not all of these parameters.

The only remarkable gross finding at necropsy was the presence of white foci on the liver of some carbaryl treated mice. The foci, generally single and adjacent to the gall bladder, were described as "tension lipidosis at the point of attachment of the falciform ligament" by the study authors, who regarded the feature as unrelated to treatment because the feature is "regularly noted in mice of [the same] age and strain". Although no supporting data were provided, the finding is considered to be of equivocal significance due to the low incidence and unconvincing dose response relationship (see Table).

There was no treatment-related tumourigenesis. The only neoplasia were single cases of benign keratoacanthoma at 10 ppm and malignant thyoma at 30 ppm, 2 cases of malignant muscular leiomyosarcoma at 300 ppm, and an example of malignant lymphoma in each of the 30 and 1000 ppm groups.

Histological examination revealed globular deposits in the upper (umbrella) cell layer of the urinary bladder epithelium, affecting many animals at 100 ppm or greater (see Table). At 100, 300 and 1000 ppm the globules appeared transparent, slightly yellow and bifringent. At 4000 ppm, the globules were smaller than at lower doses, and were red-brown in colour. The relative severity of accumulation was dose related, but there was no accompanying irritation or hyperplastic response. The study authors did not discuss the possible composition or origin of the deposits.

Conclusion:

The NOEL was 30 ppm (equal to 5.2 mg/kg bw/d), based on the presence of deposits in the urinary bladder epithelium at and above the next highest dose of 100 ppm. Under the conditions of the study, carbaryl showed no oncogenic activity.

Statistically or Biologically Significant Effects on Food Consumption, Bodyweight, Organ Weights, Gross Morphology and Histopathology in Male Mice, Study Sa 98155

	Carbaryl concentration in diet, ppm						
	0	**10**	**30**	**100**	**300**	**1000**	**4000**
Mean food consumption, wk 8 (g/d)	5.1	5.0	4.8	4.9	5.0	4.7*	4.5**
Mean bodyweight, wk 11 (g)	29.5	29.2	29.4	29.4	30.1	30.7	27.2**
Mean bodyweight, wk 25 (g)	32.3	32.1	31.7	32.4	32.8	33.9	29.8
Liver weight (g)	1.2	1.2	1.1	1.2	1.2	1.3**	1.3*
Liver:bodyweight ratio (%)	4.2	4.2	4.3	4.2	4.3	4.4	4.9**
Kidney weight (g)	0.41	0.40	0.41	0.40	0.43	0.48**	0.44
Kidney:bodyweight ratio (%)	1.49	1.46	1.49	1.47	1.50	1.61*	1.70**
Thymus weight (g)	0.034	0.032	0.031	0.030	0.030	0.037	0.025**
Thymus:bodyweight ratio (%)	0.120	0.113	0.111	0.107	0.104	0.121	0.096
Liver, white foci (n/20)	0	0	2	1	1	3	4
Urinary bladder epithelium, deposits (n/20)	0	0	0	11	20	20	20

* $p<0.05$ ** $p<0.001$

Bigot D (1999) Validation on transgenic mice – p53 knockout mice – to predict rodent carcinogenicity Study No SA 97040 Lab: Rhone-Poulenc Agro, Centre de Recherche, rue Dostoievski, Sophia Antipolis, France Sponsor: Rhone-Poulenc Agro, rue Pierre Baizet, Lyon, France Study duration: March 13, 1997 to January 6, 1999 Report date: July 21, 1999

QA and GLP statements were included but not signed. No test guidelines are applicable to the protocol. This study was submitted together with:

Carmichael NG, Debruyne ELM & Bigot-Lasserre D (1999) The p53 heterozygous knockout mouse as a model for chemical carcinogenesis in vascular tissue Lab: Rhone-Poulenc Agro, Centre de Recherche, rue Dostoievski, Sophia Antipolis, France.

Partially complete copy of manuscript published in **Environ Health Perspect 108: 61-65 (2000).**

Since the purpose of this study is to establish the sensitivity of the experimental model to chemicals that cause vascular tumours via a genotoxic mechanism, the evaluation report will concentrate on aspects involving tumourigenic activity.

In a study designed to assess the potential utility of the p53 knockout mouse for studying vascular tumour development, groups of 20 heterozygous (+/-) males (C57Bl/6Tac-[KO]*Trp*53N5-T) were gavaged daily with urethane (Sigma Chemical Co, St Louis, USA, batch 125H0318, 100% purity, 5 mL/kg bw in 0.5% methylcellulose and 0.2% Tween 80 in water) at 1, 10 or 100 mg/kg bw/d for at least 180 d. These doses were chosen on the basis of published mouse bioassay data. A negative control group of 20 (+/-) males received 250 mg/kg bw/d d-limonene (Sigma Chemical Co, St Louis, USA, batch 075H3530, 99.4% purity) by gavage using the same vehicle and dose volume. Two vehicle control groups of 20 males each were also included, one of which was p 53 heterozygous, and the other being wild type (p53 +/+) (C57Bl/Tac-[KO]*Trp*53N5-W). Animals were obtained from Taconic (Germantown, NY, USA, via Bomholtgard Breeding and Research Center AIS, Denmark) and were 8 weeks old at study commencement. Bodyweights of the p53-knockout mice ranged between 23 and 29 g, while those of their wild type counterparts were 25 to 28 g.

Care and use of the animals were stated to be in accordance with regulations of the *Guide for the Care and Use of Laboratory Animals* and *Le Guide du Journal Officiel des Communautes Europeenes L358*. Mice were housed individually within a controlled and monitored environment, and received filtered, softened tap water and AO4C P1 powder diet *ad libitum* (Usine d'Alimentation Rationelle, Villemoisson-sur-Orge, France). Animals were checked daily for clinical signs, mortality and moribundity. Bodyweights and food consumption were measured weekly.

On study d 181-184, survivors were sacrificed by exsanguination under pentobarbital anaesthesia (60 mg/kg ip) following an overnight fast. All animals were necropsied, whether killed or found dead. Adrenal gland, brain, heart, kidneys, liver, spleen, testes and thymus were weighed (at scheduled sacrifice only). In addition to macroscopic abnormalities, the following organs/tissues were fixed and embedded for light microscopic histopathological examination: adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow, brain, epididymis, oesophagus, eye and optic nerve, gall bladder, Harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx, liver, lung, lymph nodes (submaxillary, mesenteric), pancreas, pituitary gland, prostate, sciatic nerve, seminal vesicle, skeletal muscle,

skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, salivary gland, testis, thymus, thyroid (with parathyroid), tongue, trachea, and urinary bladder. Histopathological examination was performed on all tissues from all decedents and all other mice, with the exception of those in the 1 mg/kg urethane group, from which examination was restricted to the liver, lung and kidney. Significant macroscopic findings were also examined in all groups. Femoral bone marrow smears were prepared but not examined.

Bodyweight, bodyweight change and food consumption were compared between urethane exposed groups and knockout controls by Bartlett's test for homogeneity of variances. If insignificant, analysis of variance (ANOVA) was performed. When variance was significant, Dunnett's test was performed to compare test group and control means. If Bartlett's test indicated heterogeneous variances, it was followed by the Kruskal-Wallis non-parametric 1-way analysis of variance by ranks. If the Kruskal-Wallis test was significant, Mann-Whitney's test was used to compare each group to the control group.

These same parameters were compared between the wild type and knockout controls and the knockout controls and d-limonene group, by use of the F test for homogeneity of variances. If the F-test indicated homogenous variances, a t-test was performed. If variances were heterogeneous, a modified t-test was carried out. Statistical analyses were performed using SAS programs.

Using the Pathtox computer system, organ weights were compared by a combination of ANOVA and Dunnett's test when Bartlett's test indicated homogeneous variances. If variances were non-homogeneous, variables were analysed by modified t-test.

Results:

P53 knockout *vs.* wild type vehicle controls

One animal in each group of 20 died intercurrently. From wk 1 onwards, bodyweight of the p53 vehicle controls exceeded that of their wild type counterparts, with statistical significance being maintained through most of the study. At termination the knockout controls weighed 8% more than their wild type counterparts ($p<0.01$). There was a parallel trend in food consumption, which was significantly greater ($p<0.05$ or 0.01) among knockout controls than wild types at over half the weekly measured intervals. The inter-group bodyweight difference gave rise to higher mean absolute liver weight (+7%, $p<0.05$), and lower brain and kidney:bodyweight ratios (-7%, $p<0.05$) in the p53 control group.

No inter-group differences in gross necropsy findings were observed, but several histological abnormalities were more common among the p53 knockout group than wild type mice. These were:

- Midzonal hepatocyte fatty change (25% of p53 group but absent from wild type),
- Minor renal degeneration (cortical basophilic tubules) (20% of p53 group *vs.* 10% of wild type),
- Minor renal inflammation (peripelvic mononuclear infiltrates) (20% of p53 group *vs.* 5% of wild type),
- Prostatic focal epithelial hyperplasia (20% of p53 group but absent from wild type), and
- Mononuclear cell infiltration of the pancreas (25% of p53 group *vs.* 5% of wild type).

However, the genetic difference between the p53 heterozygotes and wild type mice did not give rise to any effect on formation of neoplasia during the limited time span permitted.

Urethane treatment in p53 knockout mice

Stability of the urethane suspensions was verified and achieved concentrations lay between 91 and 122% of their nominal values.

Treatment with 100 mg/kg urethane caused depression in bodyweight gain, such that the high dose group's bodyweight was 5.5% lower than knockout controls on day 85 and was reduced by 15% at termination ($p<0.05$; see Table). Feed consumption tended to be slightly lower among the treated groups than knockout controls, but there was no consistent dose relationship and statistical significance ($p<0.05$) was seldom achieved.

Only 3/20 animals from the 100 mg/kg group survived to scheduled sacrifice. Mortality occurred from wk 15 onwards, comprising 5 moribund sacrifices and 12 animals found dead. Decedents displayed hypothermia, reduced motor activity and signs of internal haemorrhage associated with vascular or thymic tumours, including red areas on the liver and gastric mucosa and fluid filled abdominal or thoracic cavities. Half the group also displayed splenomegaly and/or hepatic pallor.

By comparison, the entire 1 mg/kg group survived, while there was 1 intercurrent death from lymphoma and another from thoracic haemorrhage at 10 mg/kg. Mice receiving 1 or 10 mg/kg urethane did not show any differences from knockout controls during the in-life phase. When the 10 mg/kg group was necropsied, 8/20 animals displayed red spots on the liver, dark fluid was noted in the thoracic cavity of 2 mice, and splenomegaly was present in 2 others. The sole macroscopic observation at 1 mg/kg, was a red spot on the liver of 1 animal.

At 100 mg/kg, there was a significant ($p<0.05$) increase in liver:bodyweight ratio, but this is not considered physiologically significant in the absence of a corresponding effect on absolute liver weight. The high dose group also showed an approximate doubling of absolute spleen weight and spleen:bodyweight ratio that escaped statistical significance, and an approximate halving of absolute thymus weight and thymus:bodyweight ratio ($p<0.05$; see Table). These data must be interpreted with caution because organs from premature decedents were not weighed, and so the sample size at 100 mg/kg was only 3, compared with 18 – 20 for the remaining groups.

Histopathology revealed hepatic angiectasis (dilated vascular spaces lined by epithelial cells and filled with erythrocytes) at 10 and 100 mg/kg, and vascular neoplasia in the livers of 18/20 mice receiving 100 mg/kg (see Table). These were generally multiple, affected a large area of the liver sections, and were associated with large thrombi. In the same group, single occurrences of hemangiosarcoma of the spleen and abdominal cavity were reported, together with 1 case of cardiac hemangioma. The 10 mg/kg group showed 1 case of multiple hepatic hemangioma.

Other tumours comprised subcutaneous sarcoma and lymphoma at 10 and 100 mg/kg, and adenoma of the lung and hepatocellular carcinoma at 100 mg/kg only (see Table). The lymphomas originated within the thymus and had metastasised. Lymphoma and sarcoma are stated by the authors to occur commonly in ageing p53-deficient mice. In this study, it is possible that urethane treatment either caused these tumours directly, or hastened their development by some other mechanism. No neoplasms were present in the 1 mg/kg group.

Numerous non-neoplastic lesions were also diagnosed. At 100 mg/kg only, there were 12 animals with bilateral retinal atrophy involving complete loss of the bacillary, outer nuclear and outer plexiform layers. Fourteen mice had large areas of hepatocyte centrilobular or midzonal necrosis. The necrosis was located in areas unaffected by tumours, sometimes associated with signs of regeneration such as increased mitotic figures. Hepatic oval cell proliferation and eosinophilic foci were also recorded in this group. At 10 mg/kg, foci of hepatic clear cell alteration and hepatocytic hypertrophy were seen in 6 and 4 mice, respectively.

Generalised atrophy/involution of the thymus was noted in 6/20 of the 100 mg/kg group and in a single animal at 10 mg/kg, accompanied by cortical lymphocytosis/apoptosis at the high dose only. Diffuse atrophy of the seminiferous tubules was noted in 5/20 mice from each of the 10 and 100 mg/kg groups. Three and 17 mice from these respective groups displayed increased splenic extramedullary haematopoiesis.

d-limonene treatment – p53 knockout mice

Stability of the d-limonene suspensions was verified and achieved concentrations lay between 94 and 105% of their nominal values.

One mouse from this group died from pyelonephritis on day 56 but all others survived. Treatment with 250 mg/kg d-limonene did not cause any treatment-related changes in the behaviour, growth or other study parameters, with the exception of a slight (3-12%) decrease in food consumption (occasionally significant; $p<0.05$ or 0.01), mononuclear cell infiltration of the renal peripelvis (9 *vs.* 4/20 in knockout controls) and slight-moderate hyperplasia of the non-glandular stomach, which occurred in 17/20 animals (absent from knockout controls). The only neoplasm observed, was a prostatic adenoma present in 1 mouse.

Bodyweight, Organ:Bodyweight Ratios and Incidence of Neoplastic Disease in P53 Knockout Mice Receiving Urethane or D-Limonene

Group	Control (+/+)	Control (+/-)	Urethane 1 mg/kg/d	Urethane 10 mg/kg/d	Urethane 100 mg/kg/d	d-limonene 250 mg/kg/d
Number of animals	**19**	**19**	**20**	**18**	**3**	**19**
Terminal bodyweight (g)	33	35**	34	35	30*	33
Liver:bodywt ratio (%)	4.2	4.1	4.2	4.2	4.8	4.3
Spleen:bodywt ratio (%)	0.27	0.26	0.26	0.28	0.56	0.27
Thymus:bodywt ratio (%)	0.113	0.109	0.116	0.114	0.047*	0.109
Number of animals	20	20	20	20	20	20
Hemangioma – liver or heart	0	0	0	1	14	0
Hemangiosarcoma – liver, spleen or abdominal cavity	0	0	0	0	8	0
Lymphoma	0	0	0	1	3	0
Adenoma – lung	0	0	0	0	5	0
Sarcoma – subcutis	0	0	0	1	1	0
Hepatocellular carcinoma	0	0	0	0	4	0
Adenoma – prostate	0	0	0	0	0	1
Total tumour bearing mice	0	0	0	3	20	1

*p<0.05 **p<0.01

Conclusions:

The study design is considered adequate, and the experimental model was indeed sensitive to vascular tumourigenesis. The positive control, urethane, is genotoxic due to formation of a reactive electrophilic metabolite. The incidence of vascular neoplasms in the current study was claimed to be similar to that observed in a previous 70-week experiment (Inai *et al.*, 1991), in which B6C3F1 mice were exposed to 1, 10 and 100 mg/kg bw/d urethane in their drinking water. By contrast, vascular neoplasms did not occur among the vehicle or negative (d-limonene) control groups. There was no evidence of increased spontaneous tumour formation in p53 knockout control mice during the study.

Due to the nature of this study, a NOEL will not be assigned.

Venkatachalam S, Tyner SD, Pickering CR, Boley S, Recio L, French JE & Donehower L (2001) Is p53 haploinsufficient for tumor suppression? Implications for the p53$^{+/-}$ mouse model in carcinogenicity testing. Toxicologic Pathology 29 (suppl.), 147-154

In this published review, the authors discuss the basic biological and molecular mechanisms underlying the enhanced susceptibility of mice heterozygous for the gene coding for the p53 protein (p53+/- mice).

In response to a variety of cellular stressors, including DNA damage, hypoxia, or aberrantly activated oncogenes, p53 is activated and transcriptionally regulates an array of genes mediating cell growth and death. Activated p53 can induce cell-cycle arrest, apoptosis, differentiation or senescence, any of which can prevent the emergence of a nascent cancer cell.

The p53+/- mouse strain contains one wild-type allele, together with an inactive mutant gene coding for p53. To better characterise the biochemical basis for the enhanced sensitivity of p53+/- mice to spontaneous tumours and tumours induced by carcinogens, the authors collected tumours from these mice and used Southern blot hybridisation to investigate whether the wild-type allele remained functional or was inactivated. Over a half of the tumours retained a structurally intact wild-type allele, while in the remainder, the wild-type allele had become completely deleted. Tumours that arose at less than 18 mo of age tended to have a higher frequency of complete p53 allele loss than those arising later in the mouse life span. P53 +/- tumours that retained the wild-type allele also retained sensitivity to apoptosis following irradiation, and displayed other markers of p53 functionality.

Comparisons of fibroblasts derived from p53 +/+, p53+/- and p53-/- mouse embryos showed that the -/- genotype had the highest growth rate and saturation density, the +/+ genotype had the lowest growth rate and saturation density, while the +/- genotype was intermediate. The -/- fibroblasts showed vitually no cell cycle arrest response to ionising radiation, +/+ fibroblasts showed strong cell cycle arrest, and +/- fibroblasts showed a discernable but impeded response. Similar findings were made with respect to the apoptotic response of spleen cells from irradiated p53 +/+, p53 +/- and p53 -/- mice.

Thus, it appears that the p53 protein is "haploinsufficient"; in other words, the loss of a single copy of the wild-type allele is sufficient to impair (but not prevent) the protein's tumour suppression activity. The authors indicated that this finding was unexpected, as it had hitherto

been believed that loss of *both* copies of a tumour suppressor gene was a prerequisite for tumour formation.

Examination of tumours removed from carcinogen-treated p53+/- mice did not reveal any consistent relationship between the carcinogen's mode of action, and whether the tumours retained or lost the remaining wild-type p53 allele. Complete loss of the wild-type allele occurred in 54 of 57 thymic lymphomas from mice exposed to benzene, a genotoxic carcinogen. However, the wild-type allele became completely lost from only 3 of 13 bladder tumours from mice treated with p-cresidine, also a genotoxin. Fibrosarcomas developing in response to insertion of a transponder microchip into the skin showed intermediate levels of loss of heterozygousity.

The authors suggest that the target tissue itself may have some influence over the loss or retention of the wild-type p53 allele, and conclude that carcinogenesis in the p53 +/- mouse model is likely to involve numerous carcinogen-tissue interactions that determine the likely site of tumour origin, tumour formation latency, the oncogenic lesions responsible for tumour formation, the cell-signaling pathways affected, and whether or not the wild-type p53 allele becomes inactivated.

Debruyne E (1998) Carbaryl 52-week toxicity study in the CD1 mouse Target organs cell cycling assessment Study No. SA 97529 Lab: Rhone-Poulenc Agro, Centre de Recherche, rue Dostoievski, Sophia Antipolis, France Sponsor: Rhone-Poulenc Agro, rue Pierre Baizet, Lyon, France Study duration: December 12 – 23, 1997 Report date: December 02, 1998 (amended report)

QA: yes. GLP: OECD (1982), EC (1986), France (1990), US EPA (1989). No test guidelines apply to this special protocol.

Study design:

The objective of the study was to evaluate the possible cellular proliferation in the kidney of 10 male mice and the liver of 9 female mice that had been exposed to carbaryl for 52 weeks in a previously submitted dietary oncogenicity study (Hamada, 1993).

Formalin-fixed, paraffin-embedded kidney and liver samples were obtained from Covance (formerly Hazleton) Laboratories Inc, Vienna, Virginia USA. Four micron thick sections were prepared from these tissues from animals from the control and 8000 ppm interim sacrifice groups, and mounted on glass slides. In addition, a section of duodenum from a control rat was prepared and added to each slide beside the mouse tissue. Due to its high cell proliferation rate, the duodenum served as a positive control.

Proliferating Cell Nuclear Antigen (PCNA) was stained for, using an immunohistochemical technique. A monoclonal antibody against PCNA was applied to the deparaffinised sections, and the immunological reaction was amplified by a secondary antibody. The sections were then "submitted to a complex streptavidin-peroxydase" and the reaction revealed using the chromogen aminoethylcarbazol. Proliferating PCNA-positive cells presented a red stained nucleus, whereas non-proliferating cells had blue nuclear colouration. One thousand randomly selected hepatocytes or cortical tubular epithelial cells were counted in each section.

The results, expressed in terms of PCNA-positive cells per 1000, were not subjected to statistical analysis.

Results:

The mean number of PCNA-positive renal cortical tubular cells in 8000 ppm males (3.90/1000, SD = 2.18) was approximately 3-fold higher than in control male kidney (1.20/1000, SD = 1.75). The study authors were uncertain as to the toxicological or biological significance of the inter-group difference in the mean. Examination of the individual animal data, however, reveals that the inter-group difference arose from a generally higher positive count at 8000 ppm than among controls. Control values ranged from 0 (6 animals) to 4 (2 animals), whereas the corresponding range at 8000 ppm was 1 (2 mice) to 7 (1 mouse). Five of the 10 treated animals had counts of 5 or greater, exceeding the upper limit of the control range. The elevated mean at 8000 ppm was therefore not caused by outlying results from a few individuals, and is considered as being real and not an artefact.

In females, there was gross variation in PCNA-positive hepatocytes among the control group (mean = 4.60/1000, SD = 7.68, range = 0 to 23). By comparison, the positive staining rate among the 8000 ppm group was greater (8.33/1000, SD = 3.84) but individual data lay within a narrower range (2 to 13). The authors dismissed the findings as having no toxicological or biological significance. Again, however, examination of the individual animal data shows a difference in the underlying pattern between the two groups. The high control mean and wide range are attributable to 2 outlying mice that gave indices of 14 and 23, respectively. The remaining 8 controls all had scores of between 0 and 3. By contrast, individual scores in the 8000 ppm group were distributed more closely around the mean. Six of the 9 treated females had values of 9 to 13, inclusive.

Positive control data from the rat duodenum were not presented.

Conclusions:

The study authors concluded that there was a trend towards a minimal increase in the number of PCNA-positive cortical tubular cells in male mice treated with 8000 ppm carbaryl in the diet, compared with controls. They found there was no convincing evidence for an increase in the number of PCNA-positive liver cells in female mice exposed to carbaryl under the same conditions.

The reviewing toxicologist interprets these data as suggesting a higher amount of cellular replication in the kidney of male mice and the liver of females receiving 8000 ppm carbaryl, compared with controls. There is an apparent correlation between this parameter and Hamada's (1993b) finding of elevated incidences of renal and hepatic tumours in the 8000 ppm males and females, respectively. [Irisarri (1996, see below) found no increase in cell replication in the kidney of female mice and liver of males at 8000 ppm, concordant with the absence of renal or hepatic cancer in these groups].

While the findings are of interest, several factors detract from the reliability of the study. In particular, limited numbers of animals and time points were sampled, and there was an absence of statistical analysis or positive control readings. Interpretation of the results would have been assisted by more (particularly control) data, given that the rate of liver or kidney cell division may change with time and cause within- and between-animal variation, which has not been well characterised.

Mice & Rats

Irisarri E (1996) Carbaryl 52-week toxicity study in the rat and mouse Target organs cell cycling assessment Pathology report (post-mortem) Study No. SA 95493 Lab: Rhone-Poulenc Agro, Centre de Recherche, rue Dostoievski, Sophia Antipolis, France Sponsor: Rhone-Poulenc Agro, rue Pierre Baizet, Lyon, France Study duration: November 21 - December 08, 1995 Report date: March 18, 1996

QA: yes. GLP: OECD (1982), EC (1986), France (1990), US EPA (1989). No test guidelines apply to this special protocol.

Study design:

The objective of the study was to evaluate the possible cellular proliferation in the liver, urinary bladder and thyroid gland of rats, and the kidney and liver of mice, taken from animals that had been exposed to carbaryl for 52 weeks in previously submitted dietary oncogenicity studies (Hamada, 1993a, b).

Formalin fixed, paraffin embedded tissue samples were obtained from Hazleton Laboratories Inc, Vienna, Virginia USA. Sections used for the PCNA test were prepared from the following tissues/animals:

- Liver of 10 control and 10 treated (7500 ppm) female rats,
- Urinary bladder (urothelium) of 9 control and 9 treated (7500 ppm) male rats,
- Thyroid gland (follicle) of 9 control and 9 treated (7500 ppm) male rats,
- Liver of 9 control and 10 treated (8000 ppm) male mice, and
- Kidney (tubular epithelium) of 10 control and 10 treated (8000 ppm) female mice.

Proliferating Cell Nuclear Antigen (PCNA) was stained for using an immunohistochemical technique. A monoclonal antibody against PCNA was applied to the deparaffinised sections, and the immunological reaction was amplified by biothynile, a secondary antibody. The sections were then "submitted to a complex streptavidin-peroxydase" and the reaction revealed using the chromogen aminoethylcarbazol. Proliferating PCNA-positive cells presented a red stained nucleus, whereas non-proliferating cells had blue nuclear colouration. One thousand randomly selected cells were counted in each slide.

The results, expressed in terms of PCNA-positive cells per 1000, were not subjected to statistical analysis.

Results:

Mice

Number of PCNA Positive Cells per 1000, Mean ± Standard Deviation

Tissue	Sex	Control	8000 ppm carbaryl
Kidney (N=10)	F	0.80 ± 1.32	1.70 ± 2.21
Liver (N=9 control, 10 treated)	M	6.00 ± 11.93	4.60 ± 6.19

The study author did not consider that there was any biologically significant difference between cell cycling activity in the above groups. Given that individual animal data displayed a similar pattern in treated mice as in controls, the reviewing toxicologist agrees.

Rats

Number of PCNA Positive Cells per 1000, Mean ± Standard Deviation

Tissue	Sex	Control	7500 ppm carbaryl
Thyroid gland (N=9)	M	0.00	0.56 ± 1.67
Urinary bladder (N=9)	M	0.33 ± 0.71	3.33 ± 5.02
Liver (N=10)	F	0.60 ± 1.58	1.00 ± 2.21

The increase in mean PCNA positive thyroid cell count among 7500 ppm males was attributable to a count of 5 in a single rat. All others in this group yielded a value of zero. Cell cycling activity in the liver of 7500 ppm females was similar to that observed in controls. Most liver sections from both groups showed no PCNA-positive staining cells, with 2 or 3 animals showing counts ranging from 1-5 (controls) or 1-7 (treated group). Consequently, the reviewing toxicologist does not ascribe any significance to the slight increases in mean cell cycling activity seen at 7500 ppm. However, the study author did regard the findings as related to treatment.

By contrast, the increase in urinary bladder urothelial cell cycling in 7500 ppm males was unequivocal, was correlated with marked hyperplasia in Hamada's (1993a) study, and is regarded by the study author and the reviewing toxicologist as biologically significant.

Conclusions:

Treatment with 8000 ppm carbaryl for 52 wk did not influence cell cycling activity in the male mouse liver or female mouse kidney. This finding is consistent with the lack of treatment-related carcinogenesis reported by Hamada (1993b) in the liver of males and kidney of females.

In rats, there was a small increase in cell cycling activity in the male thyroid and female liver at 7500 ppm. Although regarded by the reviewing toxicologist as of equivocal biological significance, it should be noted that there was an elevated incidence of thyroidal adenoma and hepatic adenoma in 7500 ppm males and females, respectively, in Hamada's (1993a) 2-yr dietary experiment. The current study would have been improved by cell cycling data from animals necropsied later than 52 wk, as it could have revealed whether there is any subsequent larger increase in cell division rate that could be associated with the eventual development of neoplasms in these organs.

The 10-fold increase in cell cycling in the urinary bladder epithelium of 7500 ppm males was of clear biological significance and correlates with the hyper- and neoplastic response observed by Hamada (1993a) in the bladder of rats receiving 7500 ppm carbaryl.

Cohen SM (1995) Evaluation of the urinary bladder carcinogenicity of carbaryl in rats Unnumbered discussion paper Report date: November 2, 1995

This paper discusses the potential mechanism of toxicity underlying development of hyper- and neoplastic disease in the urinary bladder and kidney of rats during the 2-yr chronic/oncogenicity study by Hamada (1993a).

Cohen bases his presentation on the histology data in Hamada's incidence tables, which were subsequently revised in Hardisty's (1996) working group review report. The principal difference between Hardisty's findings and those of Hamada lies in the pathology reviewer's diagnosis of transitional cell hyperplasia in the urinary bladder of rats that had been treated with 7500 ppm carbaryl for 53 wk. The feature, which was not reported in the original study, persisted in some animals from this group even after a 4-wk recovery period. At study termination, Hardisty confirmed Hamada's original observations of bladder transitional cell hyperplasia, papilloma and carcinoma in the 7500 ppm group, albeit with small changes in incidence rates. Similarly, the pathology reviewer diagnosed renal pelvic epithelial hyperplasia in the 7500 ppm group at wk 53, (previously unreported), but confirmed the presence of pelvic epithelial hyperplasia and transitional cell carcinoma at 7500 ppm at study termination.

The only effect of the pathology review has been to invalidate Cohen's statement that hyper- and neoplastic lesions were not present in the urinary bladder at interim sacrifice.

At the outset of his paper, Cohen agrees with Rhone-Poulenc's position that the weight of evidence strongly suggests that carbaryl does not act via a genotoxic mechanism. He considered it more likely that the observed bladder tumours were related to increased cell proliferation in the target tissue, resulting from either an increase in cellular division or a decrease in cell death. Increased cell division could be "due to either a direct mitogenic effect on the epithelium or due to toxicity and consequent regeneration". Of these 2 possibilities, mitogenesis was more likely given the absence of treatment-related calculus formation, ulceration, necrosis or inflammation within the urinary system in studies with carbaryl. While suggesting that inhibition of apoptosis or epithelial differentiation could also potentially decrease cell death, and that more than one mechanism may be involved with any given chemical, the author concentrates on the hypothesis that carbaryl or its metabolites exerts a mitogenic effect on the bladder epithelium.

The strongest evidence in favour of Cohen's argument is drawn from his mechanistic study with another aromatic carbamate, propoxur, which has also been shown to cause urinary bladder cancer in rats (but not mice) at a high (8000 ppm) dietary dose. Using the highly sensitive method of scanning electron microscopy, Cohen *et al.* (1994) demonstrated in a 4-wk rat study, that dietary administration of 8000 ppm propoxur caused cellular proliferation that was not associated with necrosis of the bladder epithelium, formation of calculi or amorphous precipitates, or microcrystalluria.

Both carbaryl and propoxur are metabolised by 3 major pathways:

- epoxide formation with subsequent metabolism to mono- and diphenolic metabolites (with subsequent conjugation as glucuronides or sulfates);
- hydrolysis of the carbamate; and
- oxidation of the alkyl moiety.

Cohen observes the similarity between the mono- and diphenolic metabolites of carbaryl and those of propoxur and butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which also elicit proliferation within the rat urinary bladder epithelium, but do not exert a corresponding effect in mice. These compounds are excreted predominantly via the urine, and their metabolites would therefore make prolonged, direct contact with the bladder epithelium. Furthermore, Cohen states that the histological appearance of the bladder is similar following treatment with high doses of these 4 chemicals. The author believes that the monophenolic metabolites are responsible for cell proliferation, given the similarity between the diphenolic

metabolites and catechol and its analogues, which do not have an effect on the rat bladder epithelium, even at 8000 ppm in the diet.

No mechanism for the hypothesised mitogenic effect was proposed. Although the author stresses the likelihood of a localised, direct effect on the target tissue, he also notes that in Hamada's study, weight gain was depressed by greater than 10% at 7500 ppm. Cohen suggests that "an alteration of the metabolism and excretion of growth factors involved in bladder proliferation, such as epidermal growth factor or IL-6, could be greatly altered by the systemic toxicity". [In fact, terminal bodyweight of males was 65% and females was 55% of their respective controls.]

With regard to the proliferative lesions seen in the male rat kidney at 7500 ppm, Cohen also attributes the increased incidence of these to mitogenic stimulus.

The paper concludes with a brief consideration of possible human relevance of the urinary tract lesions in rats. Cohen observes that without knowing the exact mechanism involved in rats, nor the exact route of carbaryl metabolism in humans, it was impossible to predict whether the phenomenon could occur in humans. However, given that urinary tract hyperplasia/neoplasia were restricted to one species (rats) and required dietary exposure that exceeded the MTD, such lesions were unlikely at the anticipated levels of human exposure. In this respect, Dr Cohen's conclusions are both reasonable and consistent with the position taken by the Australian reviewing toxicologist in OCS's 1998 evaluation.

1.4.6 Reproduction Study

Rats

Tyl RW, Myers CB & Marr MC (2001) Two-generation reproductive toxicity evaluation of carbaryl (RPA007744) administered in the feed to CD (Sprague-Dawley) rats Study No. 65C-07407-400 Lab: Reproductive and Developmental Toxicology Laboratory, Center for Life Sciences and Toxicology, Research Triangle Institute, Research Triangle Park, NC, USA Sponsor: Aventis CropScience, Research Triangle Park, NC, USA Study duration: September 27, 1999 – November 14, 2000 Report date: May 24, 2001

QA: Yes GLP: US EPA FIFRA (1989) Test guidelines: US EPA OPPTS 83-4 and OECD (1983)

Study design

Technical grade carbaryl (Batch No. E12018008, a white to light tan powder, purity 99.1%) was received from Rhone-Poulenc Ag Co, Institute Plant, WV, USA. Dosed feeds were prepared by mixing an appropriate weight of carbaryl with 3 kg of Purina Certified Rodent Chow (No. 5002, PMI Feeds Inc, St Louis, MO, USA) and then blending the premix into batches of diet. Homogeneity and stability of the test chemical were assessed in batches of diet containing 75, 100 and 6000 ppm carbaryl. Stability analyses were performed on samples kept at room temperature for up to 11 d, and on samples stored at –20^{o}C for up to 51 d. Based on the stability data, experimental diets were prepared at least monthly and stored frozen, and feed in the feed jars was changed at least every 7 d. Regular analyses of dietary concentrations of carbaryl were performed at all doses and on control diets.

The study authors describe a range-finding study (Tyl *et al.*, 2000; not submitted) in which F0 CD rats (10/sex/dose) were exposed to carbaryl in the diet at 0, 100, 500, 2000 and 6000 ppm for 14 d and then during 14-d mating, 3-wk gestation and 3-wk lactation periods. When the F1 progeny were weaned, 10/sex/dose were exposed to the same dietary carbaryl level as their parents for a 2-wk period. F0 parental toxicity (profoundly reduced bw and feed consumption) was observed at 2000 and 6000 ppm. In the F1 generation, reduced bw and increased mortality occurred at 6000 ppm, and pup bw was reduced at 2000 ppm also. Based on these results, the target dietary concentrations for the main study were 0, 75, 300 and 1500 ppm.

In the main study, outbred CD (Sprague-Dawley) rats (Crl:CD [SD] IGS BR) were obtained from Charles River Breeding Laboratories, Raleigh, NC, USA. On arrival, the animals were 42 d old and weighed 151-175 g (M) and 126-150 g (F). During a 7-d quarantine period, 5 rat/sex were killed for assessment of serum viral antibody analysis. Four additional animals/sex were assigned as sentinels and 2/sex were killed for serology coinciding with sacrifice of the F0 and F1 parental females.

Treatment commenced with 30 F0 rats/sex/group when the animals were approximately 7 wk old and weighed 226-277 [M] or 163-204 g [F]. Thereafter, rats received their diet and water *ad libitum* throughout the study. Following a 10-wk prebreed period, F0 males and females underwent a mating period that lasted for 14 d or until vaginal sperm or copulatory plug were observed. Oestrous cyclicity was evaluated during the last 3 wk of the prebreed period and during mating. Dams were allowed to rear their young until LD 21, when the litter was weaned and at least 1 F1 pup/sex/litter (if possible) was selected randomly to produce the F2 generation. A further 3 pups/sex/litter were necropsied, while the remaining F1 pups were examined externally, killed and discarded. The selected F1 breeders were examined for vaginal patancy or preputial separation during the prebreed period, which continued until they were mated at 13-15 wk of age, under the same protocol as used for the F0 mating. Three F2 pups/sex/litter were subjected to a gross external and visceral necropsy.

During the study, rats were housed individually in solid-bottom polycarbonate cages with steel wire lids and woodchip litter. Animals were housed individually except for the mating periods, when 1 male was co-housed with 1 female. The laboratory environment was controlled and monitored. Parental F0 and F1 adults were checked twice daily for mortality, and their general condition was noted daily. Their bodyweights were recorded initially and then weekly during mating. During gestation, F0 and F1 females were weighed on GD 0, 7, 14 and 20. Dams producing litters were weighed on LD 0, 4, 7, 14 and 21, and bw gains were computed. Feed consumption was measured weekly during the prebreed period. Feed consumption of females was recorded over GD 0-7, 7-14 and 14-20 and over LD 0-4, 4-7, 7-14 and 14-21. However, the study authors noted that maternal feed consumption measurements made after LD 14 were confounded by the contribution from their pups, which were eating the diet during the last wk of lactation. Feed consumption was not measured during cohabitation.

All F1 and F2 pups were sexed at birth and examined to determine the numbers of viable and stillborn pups/litter. Litters were evaluated again for survival, bw and physical abnormalities on LD 4, 7, 14 and 21. Anogenital distance and bw were recorded from F2 pups at birth. All pups dying during lactation were necropsied. On LD 4, litters were culled 5 pups/sex, where possible. Weanlings, adults and moribund pups were sacrificed by CO_2 asphyxiation, whereas F1 and F2 pups culled on LD 4 were decapitated.

Parental F0 and F1 rats were subjected to a complete necropsy, involving examination of external surfaces, orifices, cranial cavity, carcass, external and cut surfaces of the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their organs, and cervical tissues and organs. The following organs were weighed and stored in fixative: ovaries, uterus, adrenal glands, pituitary, liver, spleen, prostate, testes, brain, kidneys, seminal vesicles and epididymides. All adult females were subjected to vaginal lavage to determine the stage of oestrus at termination. Full histopathology was performed on 10 control and 1500 ppm F0 and F1 adults, comprising the above tissues (except brain, spleen, pituitary and adrenals) together with vagina and tissues with potentially treatment-related gross lesions. Histological examination of organs showing treatment-related changes was extended to the remaining high dose and control animals, together with the 75 and 300 ppm groups. Gross lesions and reproductive tissues from unsuccessful breeders (and animals exhibiting other evidence of reduced fertility) were examined also. In addition, reproductive organs were subjected to a special examination that included sperm count, motility and morphology and enumeration of primordial ovarian follicles.

The F1 and F2 pups necropsied on LD 21 were subjected to a gross internal and external examination and their brain, spleen and thymus were weighed. Any gross lesions from these pups were retained in fixative.

Treatment groups were compared with the concurrent control group using either parametric ANOVA (if variances were homogeneous by Levene's test) or robust regression methods (REGRESS procedure of SUDAAN Release 7.5.3) that did not assume homogeneity of variance or normality. If regression tests for overall treatment group differences were significant, they were followed by individual comparisons between treated and control groups. The presence of linear trends in homogenous data was analysed by GLM procedures of SAS Release 6.12, and, when a significant treatment effect was present, pairwise comparisons between test and control groups were run by Dunnett's test. Pairwise comparisons were 1-tailed except for body and organ weight parameters, feed consumption, percent males/litter and anogenital distance.

Incidence data were analysed by Chi-Square test for independence, and by the Cochrane-Armitage test for linear trend on proportions. If the Chi-Square test was significant ($p<0.05$) then a Fisher's exact probability test was used for pairwise comparisons between the control and treated groups. Vaginal patency, preputial separation and anogenital distance were analysed by ANCOVA using bw as the covariate. For correlated data, SUDAAN software was used for analysis of overall significance, presence of trend, and pairwise comparisons to control group values. A SAS test for statistical outliers was performed on parental bw and feed consumption and on parental and weanling organ weights at necropsy. Outlying data were excluded if there was no biologically sound reason for its inclusion. For all statistical tests, the significance limit of 0.05 (1- or 2-tailed) was used as the criterion for significance.

Results

All test diets contained carbaryl at 93 to 110% of target concentrations. No carbaryl was detected in the control diet (the analytical detection limit was 2.6 ppm).

Mean achieved carbaryl doses (mg/kg bw/d) during study 65C-07407-400

Study phase	Carbaryl concentration in diet (ppm)							
	Males				Females			
	0	**75**	**300**	**1500**	**0**	**75**	**300**	**1500**
F0 adults, prebreed, wk 1-10	0	4.7	31	92	0	5.6	36	111
F0 dams, GD 0-20	-	-	-	-	0	4.8	32	96
F0 dams, LD 0-21	-	-	-	-	0	13	85	258
F0 males, postbreed, wk 1-3	0	3.5	24	70	-	-	-	-
F1 adults, prebreed, wk 1-10	0	5.8	23	124	0	6.4	27	136
F1 dams, GD 0-20	-	-	-	-	0	5.2	21	103
F1 dams, LD 0-21	-	-	-	-	0	13	51	244
F1 males, postbreed, wk 1-3	0	3.9	16	81	-	-	-	-
Overall study (F0 & F1 rats)	**0**	**4.7**	**19**	**97**	**0**	**7.1**	**37**	**143**

Analysis of the sentinels sacrificed with the F1 parental animals revealed the presence of serum antibodies to rat coronavirus/sialoacryoadenitis (RCV/SDA). Antibodies to the virus were subsequently found in a 1500 ppm F1 group female. Three other F1 females were also tested but returned negative results. None of the F1 generation had exhibited clinical signs indicitave of SDAV. The source of infection was identified as a shipment of rats that was intended for a different study and housed separately from animals in this study. The SDAV infection probably occurred around the commencement of the F1 prebreed period and resolved prior to mating. Given that the viral infection in the study population was asymptomatic, it is not considered to have adversely affected the outcome.

F0 generation

In the F0 generation, all females survived to scheduled necropsy. One 300 ppm male was found dead 60 d after study commencement, while another from this same group was withdrawn after escaping. There were no treatment-related clinical signs throughout the prebreeding period. Oestrus cycle length was slightly prolonged at 1500 ppm, but the difference from control is not considered large enough to be biologically significant, despite a significant ($p<0.05$) linear trend (see table).

Throughout the 10-wk prebreeding period, bw gain was inhibited in males at 1500 ppm, and the 1500 ppm male group had a significantly lower mean bw than controls (see table). A dose-related downward trend in male bw was evident. The effects were most prominent during the first 6 wk of the study, but were still evident 3 wk after completion of the mating period. In the 1500 ppm females, there were similar but slighter effects on bw gain that achieved statistical significance ($p<0.001$) during wk 1, and a deficit in bw that was significant ($p<0.05$ or 0.01) up to wk 7. Mean bw gain and bw were slightly lower among 300 ppm females than controls, but statistical significance was never attained.

There were parallel effects on absolute feed consumption, which was significantly reduced in 1500 ppm males throughout the prebreed period (see table) and also after the mating period, although the effect was not evident when feed consumption was adjusted for bw. Feed conversion efficiency was impaired by 16-18% in 1500 ppm males during prebreed wk 1 ($p<0.001$) and in 300 and 1500 ppm males during prebreed wk 6 ($p<0.05$). Again, females showed a similar but slighter trend. Absolute feed consumption was reduced in 1500 ppm females during prebreed wk 3 and 5, and in 300 ppm females at wk 5 ($p<0.05$ or 0.01 *vs* control, with a significant [$p<0.001$] linear trend at wk 5 only). However, there was no effect on overall feed consumption from wk 1-10, or when feed consumption was adjusted for bw. Feed conversion efficiency was impaired by 26% in the female 1500 ppm group during prebreed wk 1 ($p<0.001$ *vs* control), but not subsequently.

Carbaryl treatment did not influence the reproductive behaviour or success of males, or number of pregnancies achieved. The only remarkable clinical observation was alopecia, which was present in some treated dams during gestation but not in controls (see table). The 1500 ppm F0 dams gained significantly less weight during gestation and had depressed bw on GD 20 (see table). There was also decreased maternal absolute feed consumption (GD 14-20) and feed conversion efficiency (GD 0-20) at 1500 ppm. During lactation, F0 maternal bw was depressed at 1500 ppm but there were no treatment-related effects on bw gain, feed consumption or feed conversion efficiency. Alopecia persisted in some treated dams but remained absent from controls.

When terminated, 1500 ppm males weighed slightly but not significantly less than controls and there was a weak dose-related negative linear trend in bw (see table). Treated and control female terminal bodyweights did not show any such trend. The 1500 ppm females displayed a 10% and 14% increase (compared to control) in absolute and relative liver weights ($p<0.001$) that is considered biologically significant. However, no biological significance is attached to a 6-7% increase in mean relative kidney weight in both sexes ($p<0.05$ and <0.01), and an 11% decrease in absolute adrenal weight in females ($p<0.05$) at 1500 ppm, as these findings are probably due to decreased bw. There were no treatment-related gross findings in F0 males at necropsy but alopecia had persisted among treated females, albeit without a dose-response relationship.

Statistically and biologically significant effects in F0 rats, study 65C-07407-400 (data presented as mean ± SD or as incidence, where appropriate)

Parameter	Carbaryl concentration in diet (ppm)							
	Males				Females			
	0	75	300	1500	0	75	300	1500
No. animals	**30**	**30**	**28**	**30**	**30**	**30**	**30**	**30**
Bw gain, wk 0-10 (g)	289^^ ± 8###	288 ± 6	284 ± 5	262** ± 6	119 ± 4	119 ± 3	116 ± 3	110 ± 4
Bw, wk 10 (g)	543^ ± 9##	540 ± 7	534 ± 6	513** ± 5	304 ± 5	301 ± 4	299 ± 4	292 ± 5
Feed consumption, wk 0-10 (g/d)	27^^ ± 0.4###	27 ± 0.3	27 ± 0.4	25** ± 0.3	19 ± 0.4	19 ± 0.3	19 ± 0.3	18 ± 0.3
Terminal bw (g)	600 ± 11#	593 ± 9	588 ± 8	571 ± 9	335 ± 4	321 ± 4	328 ± 4	324 ± 4
Oestrus cycle	-	-	-	-	4.6 ±	4.4 ±	4.3 ±	4.8 ±

length, wk 1-10 (d)					0.2#	0.1	0.1	0.1
No. F0 dams pregnant	-	-	-	-	29	24	27	30
Bw gain, GD 0-20 (g)	-	-	-	-	138^^ ± 3###	130 ± 4	132 ± 3	121*** ± 3
Bw on GD 20 (g)	-	-	-	-	434^ ± 5##	422 ± 6	422 ± 6	407** ± 6
Feed consumption, GD 14-20 (g/d)	-	-	-	-	24^ ± 0.4##	24 ± 0.4	23 ± 0.3	22* ± 0.4
Feed conversion GD 0-20 (% efficiency)	-	-	-	-	31^ ± 1##	29 ± 0.6	30 ± 0.5	28** ± 0.6
Dams with alopecia, GD 0-20/LD 0-21	-	-	-	-	0/0	4/5	5/5	4/4
Bw on LD 21 (g)	-	-	-	-	342^ ± 4	328 ± 4	334 ± 4	327* ± 4

*p<0.05, **p<0.01, ***p<0.001; Dunnett's test
^p<0.05, ^^p<0.01, ^^^p<0.001; ANOVA test
#p<0.05, ##p<0.01, ###p<0.001; test for linear trend

There were no treatment-related effects on the reproductive organs of F0 females. A single 1500 ppm male had no motile sperm and 100% of its epididymal sperm were classified as abnormal ("head only"). However, this animal did not have any testicular histological abnormalities, had successfully mated, and had sired a litter of 14 live pups, all of which survived through lactation. Perhaps under the influence of this rat, group mean sperm motility was reduced at 1500 ppm (see table). There was also a concomitant increase in the proportion of abnormal sperm at 1500 ppm, together with an apparent dose-relationship and a possible effect at 300 ppm. Nevertheless, neither the 300 nor 1500 ppm groups were statistically different from control. Atrophy of the seminiferous tubule was observed in a few rats but the incidence data (2/27, 1/26, 2/23 and 1/27 at 0, 75, 300 and 1500 ppm, respectively) do not suggest a treatment-related effect on the morphology of the testis.

Sperm parameters in F0 adult males at termination, study 65C-07407-400 (data presented as mean ± SD or as incidence, where appropriate)

Parameter	Carbaryl concentration in maternal diet (ppm)			
	0	75	300	1500
No. males	**30**	**30**	**28**	**30**
Motile sperm (%)	58 ± 3#	58 ± 3	60 ± 2	52 ± 3
Progressively motile sperm (%)	46 ± 3	47 ± 2	50 ± 2	42 ± 3
Abnormal sperm (%)	1.7 ± 0.1	1.9 ± 0.3	2.6 ± 0.8	5.1 ± 3.3

F1 generation

Carbaryl did not affect the sex ratio, or growth or survival of F1 foetuses *in utero*. Pups displayed no treatment-related clinical signs during lactation. Survival of pups, however, may

have been reduced during lactation at 1500 ppm (see table), although there were no statistically significant effects on the actual numbers of live pups. There was certainly a reduction in F1 pup growth rate at 1500 ppm throughout lactation (see table), together with proportional reductions in absolute thymus and spleen weights. Relative thymus and spleen weights were unaffected. Relative male brain weight was significantly ($p<0.01$) increased at 1500 ppm. Female absolute brain weight was decreased significantly ($p<0.001$) at 1500 ppm but relative brain weight was increased ($p<0.05$). These organ weight changes are considered to be secondary to decreased bw and not indicative of target organ toxicity. No treatment-related gross abnormalities were found in premature decedents or pups necropsied at the end of lactation.

Puberty was significantly retarded in both sexes, seen as a 1.4 d delay in vaginal patency and a 2.1 d delay in preputial separation (see table). These effects were correlated with lower group mean bw.

Effects on survival, growth and puberty of F1 pups, study 65C-07407-400 (data presented as mean ± SD or as incidence, where appropriate)

Parameter	Carbaryl concentration in maternal diet (ppm)			
	0	75	300	1500
No. litters	**29**	**24**	**27**	**30**
No. pups dying during lactation	13	7	25	31
Lactational index	99 ± 0.5#	100 ± 0	98 ± 1.2	95 ± 2.8
Mean pup bw at LD 21 (g)	49^^^ ± 1###	49 ± 1	51 ± 1	43***± 1
Age at vaginal opening (d)	30.6^ ± 0.3##	31.1 ± 0.4	31.1 ± 0.3	32.0** ± 0.3
Bw (g) at puberty [F]	105^^^ ± 2###	107 ± 3	106 ± 2	93** ± 2
Age at preputial separation (d)	41.6^^^ ± 0.3###	41.5 ± 0.3	41.7 ± 0.3	43.7** ± 0.6
Bw (g) at puberty [M]	214^^ ± 3###	213 ± 3	209 ± 3	199** ± 4

Lactational index = no. surviving at 21 d/no. surviving at 4 d.
*p<0.05, **p<0.01, ***p<0.001; Dunnett's test
#p<0.05, ##p<0.01, ###p<0.001; test for linear trend
^p<0.05, ^^p<0.01, ^^^p<0.001; ANOVA test

One 1500 ppm F1 male died before the prebreed phase of the study, while a 300 ppm male died after the breeding period. There was no premature mortality among the F1 adult females. During the prebreed phase, clinical signs were restricted to alopecia (both sexes), chromodacryorrhea and sores (males only) in a few treated rats. Oestrus cycling was unaffected by treatment.

Bodyweight gain was inhibited in 1500 ppm males throughout the 10-wk prebreed period, and the bw of this group remained 30-60 g below control (see table) at each weekly measurement. Bodyweight gain of the 1500 ppm female group was significantly ($p<0.05$) inhibited during the first prebreed wk but although subsequently similar to control growth, the 1500 ppm females remained lighter than their controls throughout the prebreed phase. At 300 ppm, bw gain of

males fluctuated below and above control values but was inhibited by approximately 3% over wk 1-10. The 300 ppm female group mean bw was consistently 5-10 g lower than control throughout prebreed, but their bw gain from wk 1-10 was not reduced.

The 1500 ppm groups consumed less food than controls during the prebreed phase (especially from wk 1-7), but their bw-adjusted feed consumption was elevated (see table). Feed conversion efficiency tended to be higher in 1500 ppm females from wk 1-4 ($p<0.01$ at wk 2) but this not considered to be an adverse effect. There was no overall effect on feed conversion efficiency of males.

Carbaryl treatment did not influence the reproductive behaviour or success of F1 males, and similar numbers of control and treated F1 dams achieved pregnancy. Alopecia was more common among 300 and 1500 ppm dams than the other groups, but otherwise there were no remarkable clinical signs. Carbaryl's effects on the bw, bw gain and feed consumption of F1 dams were similar to effects on the F0 generation, comprising a clear deficit in bw gain and bw at 1500 ppm (with a possible effect at 300 ppm) and reduced feed consumption and feed conversion efficiency at 1500 ppm only (see table below).

Across the entire lactation period, there were no treatment-related effects on maternal bw changes but 300 and 1500 ppm F1 dams consistently weighed less than controls, with evidence of a dose-response relationship (see table below). However, the 300 ppm group mean bw deficit only attained statistical significance ($p<0.05$) on LD 4. Feed consumption followed a similar trend, although there was no effect on feed conversion efficiency. Alopecia persisted in 300 and 1500 ppm dams but there were no other noteworthy clinical signs.

During the 3-wk postmating phase, there were no remarkable clinical observations among males. At termination, there was a dose-related negative trend in F1 male and female bw, which was significantly lower than control at 1500 ppm and non-significantly depressed at 300 ppm (see table below). Absolute feed consumption was reduced by about 7% among 1500 ppm males ($p<0.05$ *vs* controls) but there were no effects on relative feed consumption, feed conversion or clinical observations.

When necropsied, the 1500 ppm male F1 group displayed decreased absolute brain weight ($p<0.10$), pituitary weight ($p<0.05$) and epididymis weight ($p<0.05$), together with increased relative liver weight ($p<0.01$), kidney weight ($p<0.05$) and brain weight ($p<0.05$). A slight but statistically significant ($p<0.05$) reduction in absolute brain weight occurred among 1500 ppm females. All these findings are considered to be secondary to reduced bw. There were no treatment-related gross or microscopic findings. In contrast to findings in the F0 generation, sperm evaluations were similar across the treated and control groups. The proportion of abnormal sperm ranged from 3.1% at 0 and 300 ppm to 3.4% at 75 ppm. There were no dose- or treatment-related effects on sperm motility. The incidence of atrophy of the seminiferous tubule was 1/13, 0/6, 0/9 and 3/15 at 0, 75, 300 and 1500 ppm, respectively. Female reproductive organs were unaffected.

Statistically and biologically significant effects in F1 rats, study 65C-07407-400 (data presented as mean ± SD or as incidence, where appropriate)

Parameter	Carbaryl concentration in diet (ppm)							
	Males				Females			
	0	75	300	1500	0	75	300	1500
No. animals	30	30	30	29	30	30	30	30
Bw gain, wk 0-10 (g)	401^^ ± 6###	405 ± 7	389 ± 9	371** ± 7	195 ± 5	199 ± 5	195 ± 6	194 ± 5
Bw, wk 10 (g)	532^^^ ± 7###	535 ± 9	513 ± 9	476*** ± 8	309^^^ ± 5###	314 ± 6	303 ± 6	284** ± 5
Feed consumption, wk 1-10 (g/d)	28^ ± 0.4##	28 ± 0.4	28 ± 0.4	26* ± 0.5	21^^ ± 0.4	20 ± 0.4	20 ± 0.4	19** ± 0.3
Feed consumption, wk 1-10 (g/kg bw/d)	78^^^ ± 1###	77 ± 1	78 ± 1	83** ± 1	87^^ ± 1#	86 ± 1	90 ± 1	90 ± 1
Terminal bw (g)	605^^^ ± 7###	618 ± 11	595 ± 11	548*** ± 10	343^^^ ± 6###	349 ± 4	332 ± 7	315** ± 5
No. F1 dams pregnant	-	-	-	-	29	25	26	27
Bw gain, GD 0-20 (g)	-	-	-	-	140^^^ ± 5###	137 ± 4	134 ± 3	115*** ± 3
Bw on GD 20 (g)	-	-	-	-	441^^^ ± 7###	439 ± 7	429 ± 8	392*** ± 7
Feed consumption, GD 0-20 (g/d)	-	-	-	-	24^ ± 0.9##	25 ± 0.6	25 ± 0.7	22 ± 0.4
Feed conversion GD 0-20 (% efficiency)	-	-	-	-	29 ± 1#	28 ± 0.8	28 ± 0.6	26 ± 0.6
F1 dams with alopecia, GD 0-20/LD 0-21	-	-	-	-	1/1	2/2	5/7	4/4
Bw on LD 21 (g)	-	-	-	-	348^^ ± 6###	352 ± 4	336 ± 8	324** ± 4
Feed consumption, LD 0-21 (g/d)	-	-	-	-	61^^^ ± 1###	60 ± 2	57 ± 2	51*** ± 1

Lactational index = no. surviving at 21 d/no. surviving at 4 d.
*p<0.05, **p<0.01, ***p<0.001; Dunnett's test
#p<0.05, ##p<0.01, ###p<0.001; test for linear trend
^p<0.05, ^^p<0.01, ^^^p<0.001; ANOVA test

F2 generation

There were no treatment-related effects on the sex ratio or survival of F2 foetuses during the gestation period. Although there was no effect on F2 pup birthweight, a significant deficit in bw was evident at 1500 ppm from LD 4 onwards, persisting until weaning (see table below). Pup

survival was also impaired. There was a deficit in the lactation index at 1500 ppm, with a slighter shortfall in pup survival at 300 ppm (see table below). Statistical significance was not achieved in this parameter, but the finding is consistent with reduced lactation index in the F1 generation. There was also a dose-related reduction in the number of live pups/litter on LD 4, and an increased number of pup deaths at 300 and 1500 ppm.

Anogenital distance at birth was significantly reduced in 1500 ppm male pups (see table below) but not at lower doses or among female pups. When the measurement was adjusted for bw and analysed by ANCOVA, there was a significant ($p<0.01$) negative dose-related trend but none of the treated groups was significantly different from control in pairwise comparisons. Hence, the reduction in anogenital distance was probably associated with reduced pup bw and is not considered to arise from interference with the sexual development of male foetuses. On LD 7, brown crusty material was present on the noses of many treated pups, but otherwise the offspring did not show remarkable clinical signs. The time taken to reach puberty was not measured in the F2 generation, as pups were killed on LD 21.

At necropsy, many of the premature decedents showed evidence of failure to feed. Two female 1500 ppm pups from the same litter had a gross visceral abnormality consisting of an extra blood vessel arising from the iliac artery and terminating on the left kidney. Absolute thymus, spleen and brain weights were statistically or biologically significantly reduced at 1500 ppm. Relative spleen and thymus weights were unaffected. Relative brain weights were increased at 1500 ppm. However, these organ weight and organ:bw ratios reflected the lower pup bw at 1500 ppm and are not considered indicative of target organ toxicity.

Effects on survival, growth and anogenital distance of F2 pups, study 65C-07407-400 (data presented as mean ± SD or as incidence, where appropriate)

Parameter	Carbaryl concentration in maternal diet (ppm)			
	0	75	300	1500
No. litters	**29**	**25**	**26**	**27**
No. live pups/litter, LD 4	15.4^^ ± 0.4##	13.9 ± 0.7	12.7* ± 0.9	12.5** ± 0.6
No. pups dying during lactation	16	15	57	67
Lactational index	99 ± 0.8	99 ± 0.7	94 ± 4	90 ± 4
Anogenital distance at birth (mm) [M]	2.11^^^ ± 0.02###	2.17 ± 0.03	2.12 ± 0.03	2.00* ± 0.02
Anogenital distance at birth (mm) [F]	0.96 ± 0.01	1.01 ± 0.02	1.00 ± 0.02	0.96 ± 0.01
Mean pup bw at LD 21 (g)	50.9^^^ ± 0.7###	52.3 ± 0.8	49.7 ± 1.5	40.4*** ± 1.3

Lactational index = no. surviving at 21 d/no. surviving at 4 d.
*p<0.05, **p<0.01, ***p<0.001; Dunnett's test
#p<0.05, ##p<0.01, ###p<0.001; test for linear trend
^p<0.05, ^^p<0.01, ^^^p<0.001; ANOVA test

Conclusions

The NOEL for effects on the parental generations was 75 ppm (approximately 4.7 mg/kg bw/d), based on the following findings at and above the next highest dose of 300 ppm: decreased bw gain, bw, feed consumption and conversion efficiency in F0 and F1 adults of both sexes, combined with depression in gestational bw and lactational bw and feed consumption in F1 females. The NOEL for effects on pups was also 75 ppm, based on increased mortality during lactation of the F2 litters at and above 300 ppm.

The delays in achieving puberty and decreased anogenital distance occurring at 1500 ppm are considered to be secondary to decreased bw, and do not represent any specific effect on sexual development.

Comment

The data presented do not provide any evidence that the SDAV infection occurring near the start of the F1 prebreed period had any effect on the results, although the possiblity cannot be excluded.

1.4.7 Developmental Studies

Rats

Repetto-Larsay M (1998): Carbaryl Developmental toxicology study in the rat by gavage Study No: SA 98070 Lab: Rhone-Poulenc Agro, Centre de Recherche, rue Dostoievski, Sophia Antipolis, France Sponsor: Rhone-Poulenc Agro, rue Pierre Baizet, Lyon, France Study duration: February 28 – July 24, 1998 Report date: October 21, 1998

QA: yes. GLP: OECD (1982), EC (1986), France (1990), US EPA (1989), Japan (1984). Test guideline: US EPA 712-C-98-207 (1998), OPPTS 870.3700

Study design:

Carbaryl (Rhone-Poulenc, Institute Plant, WV USA, batch no 208 115 110, purity 99%) was suspended in aqueous methylcellulose 400 at 0.5% w/v and administered by gavage to mated female CD strain rats (Sprague-Dawley Crl: CD (SD) BR, obtained from Charles River Laboratories, St Aubin les Elbeuf, France, initial bw 241 – 304 g). Groups of 25 animals received 0, 1, 4 or 30 mg/kg bw/d of the test chemical as single daily doses on d 6 – 20 inclusive of presumed gestation, at a volume of 10 mL/kg bw. The doses were chosen on the basis of a range finding study (not submitted).

Animals were housed individually in suspended stainless steel mesh cages under standard laboratory conditions in a controlled and monitored environment. They had free access to pelleted diet (Usine d'Alimentation Rationelle, Villemoisson-sur-Orge, France) and filtered tap water.

Dams were examined daily for signs of illness, and their bodyweights were recorded on d 0, then daily between gestation day (gd) 6 and 21. Food consumption was recorded over d 0 – 6 and daily from gd 6 – 20.

On gd 21, surviving dams were killed by CO_2 inhalation and subjected to macroscopic examination of the visceral organs. The uterus, oviducts and ovaries were dissected out, weighed and the following parameters recorded: numbers of implantations, corpora lutea, early and late resorptions and live and dead foetuses; foetal sex; weight of placenta and individual foetus weights.

Viable foetuses were killed by sc injection of 0.02 mL sodium pentobarbital and examined externally. Approximately half of them from each litter were immersed in Bouin's fluid for internal examination following freehand sectioning, while the remainder were eviscerated, placed in ethanol, stained by the method of Staples and Schnell, and subjected to skeletal examination.

Using SAS programs, results of maternal bw and bw changes, food consumption, foetal bw and placental weight were compared between the treated and control groups by Bartlett's test for homogeneity of variance followed by ANOVA (if variances were homogeneous) and Dunnett's test if ANOVA was significant. When variances were heterogeneous, Kruskal-Wallis non-parametric ANOVA was applied followed (if significant) by the Mann-Whitney test. Litter data were analysed by Kruskal-Wallis non-parametric ANOVA, followed (if significant) by the Mann-Whitney test.

Results (maternal):

The homogeneity and stability of the test chemical were verified by HPLC. Achieved carbaryl concentrations in the vehicle were 93 – 100% of their nominal value.

No premature mortality occurred. Eighteen/25 dams from the 30 mg/kg group had at least one occurrence of increased salivation within 20 min of dosing. The feature resolved approximately 1 h post-treatment and was mainly observed from gestation d 14 to 20. The high dose group also showed significantly ($p<0.01$) depressed food consumption throughout the dosing period and a transient loss of bodyweight at the commencement of dosing. Although the 30 mg/kg dams subsequently gained weight at a similar rate to the other groups, there was an 8% (but statistically insignificant) deficit (*vs.* control) in terminal bodyweight, and a significant ($p<0.01$) reduction in cumulative gross and net (without uterus) bodyweight gain and terminal bodyweight (see Table). However, there were no treatment-related maternal abnormalities at necropsy.

Results (foetal):

All dams achieved pregnancy and implantation and pre-implantation loss rates were comparable between the treated and control groups. Foetal survival and sex ratio were not compromised but there was evidence of foetotoxicity at 30 mg/kg. This was seen as a 13% (but statistically insignificant) reduction in gravid uterine weight and a significant ($p<0.01$) deficit in foetal bodyweight (see Table). Compared with the control group, the incidence of runts (foetuses weighing 25% less than the control mean) was increased at all doses. Given the correlation with reduced mean foetal bodyweight at 30 mg/kg, the reviewing toxicologist considers runting to be treatment-related at the high dose but not at 1 or 4 mg/kg. Delayed ossification of the spinal vertebrae and paw were also observed at the high dose (see Table). However, there were no treatment-related visceral anomalies or malformations.

Statistically and Biologically Significant Findings in Pregnant Rats and Foetuses, Study Sa 98070 (Mean ± SD, or Incidence)

Dose (mg/kg bw/d)		0	1	4	30
MATERNAL FINDINGS					
Terminal bodyweight (g)		433 ± 23	439 ±25	435 ±25	397 ± 25
Bodyweight gain d 6 –21 (g)		133 ± 18	138 ± 16	132 ± 16	97 ± 18**
Net terminal bodyweight (g)		325 ± 20	329 ± 22	327 ± 21	303 ± 20**
Net bodyweight gain d 0 – 21 (g)		58 ± 15	61 ± 14	58 ± 14	36 ± 13**
Gravid uterus weight (g)		108 ± 14	110 ± 12	109 ± 13	94 ± 19
Food consumption d 18-21 (g/d)		29 ± 3.3	31 ± 2.6	29 ± 2.4	25 ± 2.3**
FOETAL FINDINGS					
Mean body-weight (g)	F	5.24 ± 0.29	5.20 ± 0.30	5.25 ± 0.34	4.87 ± 0.42**
	M	5.56 ± 0.32	5.56 ± 0.27	5.46 ± 0.33	5.10 ± 0.44**
Runts		0/377	3/389	3/377	8/359
Sternbra 5: incomplete ossification		39/196	51/201	47/196	67/186
Cervical centrum 4–6: unossified		83/196	74/201	68/196	110/186
Cervical centrum 7: unossified		9/196	8/201	11/196	19/186
Metatarsal 1: unossified		12/196	20/201	23/196	34/186

*p<0.05 **p<0.01

Conclusions:

The NOEL was 4 mg/kg bw/d, based on maternotoxicity (salivation and depressed food consumption and bodyweight gain) and foetotoxicity (reduced foetal bodyweight and delayed ossification) at the highest dose of 30 mg/kg bw/d. Carbaryl was not teratogenic under the conditions of the study.

Rabbits

Tyl RW (1999) Dose range-finding study for the developmental toxicity evaluation of carbaryl administered by gavage to New Zealand White rabbits Study No: Rb98-CAR 1 Lab: Reproductive & Developmental Toxicology Laboratory, Center for Life Sciences and Toxicology, Research Triangle Institute, Research Triangle Park, NC USA Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC USA Study duration: September 15 – October 14, 1998 Report date: March 6, 1999

QA: yes. GLP: no

Study design:

Carbaryl (Rhone-Poulenc, Institute Plant, WV USA, batch no 208 115 110, purity 99%) was suspended in aqueous methylcellulose at 0.5% w/v and administered by gavage to mated female

NZW strain rabbits (obtained from Covance, Denver, COL USA, age unstated, initial bw approximately 3.4 kg). Groups of 6 animals received 0, 3, 7.5, 20, 50 or 100 mg/kg bw/d of the test chemical as single daily doses on d 6 – 29 inclusive of presumed gestation, at a volume of 2 mL/kg bw.

Animals were housed individually in suspended stainless steel cages under standard laboratory conditions in a controlled and monitored environment. They were fed #5322 Purina Certified Rabbit Chow, which was rationed at 65 g/doe during the first 24 h, at 125 g/doe on gestation day (gd) 2, and *ad libitum* thereafter. Tap water was available throughout the study.

Does were examined daily for signs of illness prior to the dosing period and twice daily from d 6 to d 29, with an observation being conducted 1 – 2 h post-treatment. Their bodyweights were recorded on gd 0, 29 and 30, and each 3 d between d 3 and 27. Food consumption was recorded over d 3 – 6, over 72 h intervals from gd 6 – 27, and on d 27 – 29 and 29-30. On gd 28, 1 h post-dosing, a 3 mL blood sample was collected from the central ear artery of each animal. Plasma and RBC cholinesterase activity were measured spectrophotometrically by a modification of the method of Ellman *et al*.

On gd 30, surviving dams were killed by iv injection of sodium pentobarbital and subjected to macroscopic examination of the visceral organs. The body, liver and uterus were weighed and the following parameters recorded: numbers of implantations, corpora lutea, resorptions and live and dead foetuses; foetal sex; and individual foetus weights. Viable foetuses were killed by ip injection of sodium pentobarbital and examined externally.

Results (maternal):

Actual concentrations of carbaryl in the dosing mixture were verified by HPLC and lay between 95 and 110% of the target levels.

There were no treatment-related clinical signs or unscheduled deaths. A control dose was withdrawn due to a misdirected dose, while another from this same group was withdrawn after early delivery.

Maternal food consumption was unaffected. There was a significant ($p<0.05$) trend towards decreasing bodyweight gain over the dosing period but no pairwise comparisons between controls and treated groups achieved significance, even though mean values were reduced by about 20% at 50 and 100 mg/kg. A parallel (but non-significant) trend occurred in net maternal bodyweight change when corrected for gravid uterine weight (see Table). However, group mean bodyweights, gravid uterus weights and liver weights did not vary enough to reach statistical or biological significance. There were no findings at post-mortem examination.

Data on plasma and RBC ChE activities were presented for the control and high dose groups only. At 100 mg/kg, ChE activity was inhibited by 20% in RBC and 59% in plasma ($p<0.05$) relative to control values.

Results (foetal):

At termination, there were 4 control litters with viable foetuses, 5 litters at 3 mg/kg, and 6 litters at each of the higher doses. Treatment did not compromise foetal survival, but there was a near significant ($p=0.0506$) trend towards dose related depression in foetal bodyweight, attributable to

a 16% reduction (*vs.* control) at 100 mg/kg (see Table). However, this may not have been caused by treatment because mean litter size at 100 mg/kg was higher than control (8.7 kittens *vs.* 7.3). The only developmental abnormalities observed were spina bifida and clubbed limb in a single 50 mg/kg foetus, and one case of umbilical hernia at 100 mg/kg.

Statistically and Biologically Significant Findings in Pregnant Rabbits and Foetuses, Study Rb98-Car 1 (Mean ± SD, or Incidence)

Dose (mg/kg bw/d)	0	3	7.5	20	50	100
MATERNAL FINDINGS						
Bodyweight gain over gestation (g)#	597±112	632±135	552±32	598±121	465±34	473±81
Net bodyweight change over gestation (g)	48±145	4±105	-35±30	20±81	-59±61	-94±61
Plasma ChE activity (mU/mL)	199±26	ND	ND	ND	ND	82±5*
RBC ChE activity (mU/mL)	1266±115	ND	ND	ND	ND	1014±126
FOETAL FINDINGS						
Mean bodyweight (g)	54±1	50±1	52±2	52±2	51±3	45±1

ND = no data *p<0.05 #significant trend (p<0.05)

Conclusions:

There is considered to be some possible evidence of maternotoxicity at 50 and 100 mg/kg bw/d, seen as reduced gain in gross and net bodyweight. Inhibition of maternal plasma and RBC ChE activity was biologically significant at 100 mg/kg bw/d. The apparent depression in foetal bodyweight at 100 mg/kg is of equivocal significance. Due to their low incidence, the few observed external foetal malformations and variations are not ascribed to treatment.

As this is a range finding study, a NOEL will not be set.

Tyl RW, Marr MC & Myers CB (1999) Developmental toxicity evaluation (with cholinesterase assessment) of carbaryl administered by gavage to New Zealand White rabbits. Study No: 65C-7297-200/100 Lab: Reproductive & Developmental Toxicology Laboratory, Center for Life Sciences and Toxicology, Research Triangle Institute, Research Triangle Park, NC USA Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC USA Study duration: November 17, 1998 – March 4, 1999 Report date: June 3, 1999

QA: yes. GLP: US EPA (1989) Test guideline: US EPA 83-3

Study design:

Carbaryl (Rhone-Poulenc, Institute Plant, WV USA, batch no 208 115 110, purity 99%) was suspended in aqueous methylcellulose at 0.5% w/v and administered by gavage to mated female NZW strain rabbits (obtained from Covance, Denver, COL USA, aged approximately 5 mo, initial bw 2.5 – 5 kg). Groups of 22 animals received 0, 5, 50 or 150 mg/kg bw/d of the test chemical as single daily doses on d 6 – 29 inclusive of presumed gestation, at a volume of 2 mL/kg bw. The doses were chosen on the basis of the above range finding study.

Animals were housed individually in suspended stainless steel cages under standard laboratory conditions in a controlled and monitored environment. They were fed #5322 Purina Certified Rabbit Chow, which was rationed at 65 g/doe during the first 24 h, at 125 g/doe on gestation day (gd) 2, and *ad libitum* thereafter. Tap water was available throughout the study.

Does were examined daily for signs of illness prior to the dosing period and twice daily from d 6 to d 29, with an observation being conducted 1 – 2 h post-treatment. Their bodyweights were recorded on gd 0, 29 and 30, and each 3 d between d 3 and 27. Food consumption was recorded over d 3 – 6, over 72 h intervals from gd 6 – 27, and on d 27 – 29 and 29-30. On gd 25, approximately 1 h post-dosing, a 3 mL blood sample was collected from the central ear artery of each animal. Plasma and RBC cholinesterase activity were measured spectrophotometrically by a modification of the method of Ellman *et al*.

On gd 30, surviving does were killed by iv injection of sodium pentobarbital and subjected to macroscopic examination of the visceral organs. The body, liver and uterus were weighed and the following parameters recorded: numbers of implantations, corpora lutea, resorptions and live and dead foetuses; foetal sex; and individual foetus weights.

Viable foetuses were killed by ip injection of sodium pentobarbital and examined externally and for visceral and skeletal morphology. Approximately half of the foetuses from each litter were decapitated. Heads were immersed in Bouin's fluid for internal examination following freehand sectioning.

Quantitative continuous data (such as bodyweights, food consumption or ChE activity) were analysed by Bartlett's test for homogeneity of variance followed by ANOVA (if variances were homogeneous) and Dunnett's test if ANOVA was significant. When variances were heterogeneous, Kruskal-Wallis non-parametric ANOVA was applied, followed (if significant) by the Mann-Whitney U-test. Jonckheere's test was used to identify significant dose response trends for non-parametric continuous data. Nominal scale measures were analysed by the Chi-Square test and by the Cochran-Armitage test for Linear Trend on Proportions. When Chi-Square revealed significant ($p<0.05$) differences among groups, a 2-tailed Fisher's Exact Probability test was used for pairwise comparisons between each treated group and the control group.

Results (maternal):

Actual concentrations of carbaryl in the dosing mixture were verified by HPLC and lay between 101 and 115% of the target levels.

The only treatment-related clinical sign was bodyweight loss in a few of the 150 mg/kg does. One doe at each of 0 and 5 mg/kg and 2 does at 50 mg/kg were found dead on gd 29 – 30. Neither the pattern of mortality nor autopsy findings are suggestive of a test-chemical related effect. Two further controls were withdrawn due to a misdirected dose. Maternal food

consumption was elevated by approximately 20% at 50 mg/kg ($p<0.05$), both on an absolute basis and relative to bodyweight. However, the observation is attributed to chance because no similar finding was made at the low and high doses within this study, or at any dose in the range finding study.

All groups showed transient weight loss of 100 – 150 g between gd 0 and 3 but regained weight during the next 3 d. Subsequently, there was a highly significant ($p<0.05 – 0.001$) dose related trend towards decreasing bodyweight gain during the dosing period. The 150 mg/kg group lost weight over gd 6 – 9, and displayed significantly ($p<0.01$) depressed cumulative weight gain over the dosing and entire gestation periods. Group mean bodyweights did not vary enough to reach statistical significance, but there was a dose related trend towards a reduction in this parameter on d 30 (see Table). When corrected for gravid uterine weight, maternal net bodyweight loss over the entire gestation period was nearly 3-fold higher at 150 mg/kg than among controls, but the statistical comparison between these groups did not yield significance (see Table).

Plasma and RBC ChE activities were inhibited dose relatedly at 50 and 150 mg/kg ($p<0.01$). At the mid and high doses, respectively, inhibition amounted to approximately 46 and 68% in plasma and 19 and 29% in erythrocytes.

One doe from each of the control and 50 mg/kg groups failed to achieve pregnancy, a further 50 mg/kg doe resorbed its entire litter, and abortion or premature delivery occurred in 1 control and 2 of the 150 mg/kg does. At term, there were 18, 21, 18 and 20 litters at 0, 5, 50 and 150 mg/kg, respectively, no significant inter-group differences in gravid uterine weights, and no treatment-related maternal autopsy findings.

Results (foetal):

Treatment did not compromise foetal survival or sex ratio, but there was a significant ($p<0.01$) depression in foetal bodyweight at 150 mg/kg (see Table). Also present at this dose (but not at 0, 5 or 50 mg/kg), were some visceral and skeletal malformations, involving agenesis of the bile duct and/or gall bladder, and sternebral fusion. Ventricular enlargement, a visceral variation, was present at 50 and 150 mg/kg (see Table).

Historical control (HC) data from the study laboratory were provided, encompassing 1503 foetuses from 9 experiments conducted between 1993 and 1997. The incidences of bile duct and gall bladder agenesis at 150 mg/kg exceed the HC mean and range. A treatment-related effect is considered unlikely, however, given that only 2 foetuses were affected, one of which had no bile duct or gall bladder. The sternebral fusion and ventricular enlargement seen in the current study are also probably not treatment-related, because they lay within the HC range and/or mean.

Statistically and Biologically Significant Findings in Pregnant Rabbits and Foetuses, Study Rti 65c-7297-200 (Mean ± SD, or Incidence)

Dose (mg/kg bw/d)	0	5	50	150
MATERNAL FINDINGS				
Terminal bodyweight (kg)	3.82±0.08	3.82±0.08	3.71±0.09	3.69±0.08
Bodyweight gain d 6 –29 (g)##	443±55	396±33	334±36	208±33**

Dose (mg/kg bw/d)	0	5	50	150
Net bodyweight change d 0 – 30 (g)#	-82±38	-70±43	-58±48	-220±44
Gravid uterus weight (g)	611±25	595±25	511±41	546±15
Plasma ChE activity (mU/mL)♦	211±13	183±7	114±7**	67±5**
RBC ChE activity (mU/mL)♦	1083±31	1019±32	879±38**	796±33**
FOETAL FINDINGS				
Mean bodyweight (g)#	51.6 ± 1.1	50.5 ± 0.8	50.6 ± 1.6	46.4 ± 1.3**
Bile duct: agenesis	0/153 HC inc: 0/1503	0/174	0/137	1/171 (0.6%)
Gall bladder: agenesis	0/153 HC inc: 1/1503 (0.07%) HC range: 0 – 0.46%	0/174	0/137	2/171 (1.2%)
Sternebral fusion	0/153 HC inc: 9/1503 (0.6%) HC range: 0 – 1.2%	0/174	0/137	2/171 (1.2%)
Ventricular enlargement	0/153 HC inc: 39/1503 (2.6%) HC range: 0 – 10.3%	0/174	1/137 (0.7%)	3/171 (1.8%)

*p<0.05 *vs.* control **p<0.01 *vs.* control
significant linear trend (p<0.01) ##significant linear trend (p<0.001)
♦significant dose related trend (p<0.001)

Conclusions: The NOEL for maternal effects was 5 mg/kg bw/d, based on plasma and RBC ChE inhibition at and above the next highest dose of 50 mg/kg bw/d. The NOEL for foetotoxicity was 50 mg/kg bw/d, based on depressed bodyweight at the highest dose of 150 mg/kg bw/d. Carbaryl is considered not to have been teratogenic under the conditions of the study.

1.4.8 Neurotoxicity Studies

Rats

Robinson K & Broxup B (2001a) A developmental neurotoxicity study of orally administered carbaryl, technical grade, in the rat. Final report amendment number 1. Study No: 97391 Lab: ClinTrials BioResearch Ltd, Senneville, Quebec, Canada. Sponsor: Aventis CropScience, Mississauga, Ontario, Canada Study duration: Not stated Report date: July 6, 2001

And

Robinson K & Broxup B (2001b) A developmental neurotoxicity study of orally administered carbaryl, technical grade, in the rat. Final report amendment number 2. Study No: 97391 Lab: ClinTrials BioResearch Ltd, Senneville, Quebec, Canada. Sponsor: Aventis CropScience, Mississauga, Ontario, Canada Study duration: Not stated Report date: July 10, 2001

QA: Yes GLP: US EPA 40 CFR Part 160, OECD (C[81]30) & JMAFF (59 NohSan No. 3850) Test Guideline: US EPA Subdivision F, Hazard Evaluation: Human and Domestic Animals, Addendum 10 - Neurotoxicity Series 83.6
[Main] study design

This report is an amendment to Study 97391 by the same authors, which was evaluated by OCS in 1998. In the original study, pregnant female rats were gavaged with carbaryl from GD 6 to 10 d post-partum at doses of 0, 0.1, 1.0 or 10 mg/kg bw/d. From a subset of the treatment and control groups, RBC, plasma and whole blood ChE activity were measured prior to dosing on GD 6, and 1 h post-dosing on GD 6, 15 and 20, and on post-partum days 4 and 10. Brain ChE activity was measured at termination on post-partum d 10. Dams from the various main study groups were assessed by functional observational battery (FOB) at intervals throughout gestation and weaning. After post-partum d 21, a special histopathological assessment of the central and peripheral nervous systems of control and 10 mg/kg dams was undertaken. During the lactation period, pups were assessed for viability, malformations and growth, and were also examined by FOB. Sub-sets of pups were sacrificed 11 d post-partum for brain weight measurement and neurohistological examination (control and 10 mg/kg groups only). The development of the remaining pups was followed until an age of approximately 60-65 d, when behavioural assessment was performed again. Neurohistology was performed on these pups when they had attained 70 d of age.

[Main] study results

Maternal bw gain was impaired at 10 mg/kg, between GD 6 and 9. Clinical signs of neurotoxicity occurred in the 10 mg/kg dams throughout gestation, comprising ataxia, hypotonic or impaired gait, tremors and pupillary constriction. There was depression in RBC, plasma and whole blood ChE activity at 10 mg/kg on GD 20, which persisted up to 10 d post-partum. A similar depression in brain ChE activity was evident on d 10 post-partum. The maternal NOEL was 1 mg/kg bw/d, based on reduced bw gain, autonomic effects, tremors and ChE depression at the highest dose of 10 mg/kg bw/d.

Carbaryl did not influence pup birth weight, growth and development, behaviour, motor activity, passive avoidance and startle response throughout lactation. There were also no treatment-related gross pathological findings among pups dying during lactation, or at neurohistological examination on d 11 post-partum. Whole and regional brain weights were unaffected. Compared with controls, there were statistically significant ($p<0.05$, <0.01 and <0.001) differences in the size of the forebrain and cerebellum of the 10 mg/kg group at 11 and 70 d of age. The right forebrain of 10 mg/kg males was enlarged at d 10 but reduced in size on d 70, without any concomitant change in the left forebrain, or among females at either time point. Findings within the cerebellum were also contradictory. At 10 d, bilateral shrinkage was observed in 10 mg/kg females, whereas enlargement was recorded in males. At 70 d, females showed bilateral enlargement of the cerebellum, while males had enlargement of the right (but not left) cerebellum. Due to their inconsistency, these changes from control values were not considered to be biologically significant by the Australian reviewing toxicologist. Carbaryl was therefore judged to have had no effects on foetal survival or growth, or development or behaviour of pups under the study conditions.

Amendment numbers 1 & 2

Amendments 1 and 2 were closely similar, except that the introduction to Amendment 2 stated that the additional work described below had been carried out in response to the US EPA review of the main study (No. 97391).

According to the study authors, the US EPA indicated a possible relationship to treatment in respect of a bilateral decrease in the length of the cerubellum, accompanied by a statistically non-significant 5% decrease in cerebellar weights in 11 d old female offspring of dams treated at 10 mg/kg bw/d. A bilateral increase in the width of the cerebellum in 70 d old female offspring from the same group was also considered as being possibly related to treatment. Furthermore, some unidentified forebrain measurements may have been affected. The US EPA was stated to have recommended that additional morphometric measurements be performed on the 0.1 and 1.0 mg/kg groups to better support the NOEL and that the thickness of the cellular layers in the cerebellum be more fully described. In the event, the study laboratory re-evaluated data from the 10 mg/kg groups but not from offspring whose dams received carbaryl at 0.1 and 1.0 mg/kg bw/d.

Following a methods development study (Hamelin & Yipchuck, 2001; submitted but not reviewed), supplementary morphometric analyses were carried out on the forebrain and cerebellum in the 11- and 70-d old offspring from the control group and dams receiving 10 mg carbaryl/kg bw/d. The measurements were:

- Cerebellum: external granular layer thickness (lobule 5) at 11 d old (both sexes);
- Cerebellum: internal granular layer thickness (lobules 4 and 5) at 11 d old (both sexes);
- Cerebellum: lobule 5 base thickness at 11 d old (both sexes);
- Cerebellum: lobule 5 base thickness at 70 d old (both sexes);
- Cerebellum: granular layer thickness at 70 d old (both sexes);
- Forebrain: thickness of parietal cortex at 11 d old (males only);
- Forebrain: thickness of frontal cortex at 70 d old (males only).

Group variances for measurements were compared using Bartlett's test. The differences between group variances were not significant ($p>0.001$), and Dunnett's test was used to compare the control and treated group (10 mg/kg bw/d) for significant differences. The study authors stated that cerebellar measurements were focussed on the granular cell layer because some op compounds have been shown to cause degeneration of granular cells, resulting in atrophy of the cerebellum. It was noted also that there is no external granular layer in the normal mature cerebellum, due to inward migration of the external granular cells as the brain matures. Hence, measurements of the external granular layer were performed only in 11-d old pups.

The findings of these two supplementary studies were entirely negative with respect to all measured parameters. There were no statistically or biologically significant differences between control offspring and those from the 10 mg/kg group, at either 11 or 70 d of age. The study authors also reported that there were no morphological differences between the cerebellar structure treated and control groups.

Conclusions

The additional histomorphometric evaluation does not change the original conclusion that there were no neurotoxic or developmental effects on pups at the highest dose (10 mg carbaryl/kg

bw/d). The maternal NOEL remains at 1 mg/kg bw/d (based on reduced bw gain, autonomic effects, tremors and ChE depression at the highest dose).

1.4.9 Human Studies

Field Studies

Merricks DL (1997a) Carbaryl applicator exposure study during application of Sevin 5 Dust to dogs by the non-professional. Study Nos: 1517 (Lab) and 10565 (Sponsor) Labs: Agrisearch Inc, Frederick, MD USA; Rhone-Poulenc Ag Company, Research Triangle Park, NC USA; and Morse Laboratories Inc, Sacramento, CA USA Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC USA Study duration: February 17 to August 22, 1997 Report date: August 22, 1997

QA: yes. GLP: US EPA 40 CFR Part 160 (1989) Test guideline: US EPA Subdivision U, Applicator Exposure Monitoring Series 231 and 232, Occupational and Residential Exposure Test Guidelines Group A, Application Exposure Test Guidelines

Study design:

This study assessed carbaryl inhalational and dermal exposure among a group of volunteers, under conditions intended to simulate use of a home veterinary insecticide in compliance with label directions.

The study group consisted of 20 male and non-pregnant female volunteers aged 19 – 45 y who had given informed consent prior to participation. The volunteers' mean weight was 78.8 kg and mean height was 1.73 m. None of the study group were professional pet groomers.

The product used was Sevin Carbaryl Insecticide 5 Dust (Manufactured by Solaris Group/Aerofil Technology Inc, St Louis, MO USA, lot 20187A), a 5.38% active constituent powder formulation registered by US EPA for control of fleas and ticks on dogs and cats. The non-active constituents present in the product were not stated. The product was applied directly from the shaker top dispenser in which it was packaged.

Forty replicate exposures were performed. Twenty exposures were carried out with the applicator wearing household type latex gloves, while the remainder were performed without gloves. Each volunteer conducted one replicate with gloves followed by a replicate without gloves. Throughout the procedure, volunteers wore cotton long sleeved shirts and long pants over a cotton whole body dosimeter. Their footwear was covered with lab booties before entry into the application area. Breathing zone monitoring was performed using a personal air sampling pump operating at 2 L/min with an OVS sorbent tube containing XAD-2 resin.

The exposure phase of the study was undertaken in a heated, cement floored external garage attached to a dog pound, where the study animals were housed. The dogs used in the study were chosen randomly and were of various breeds, weighing between 1.8 and 57 kg (mean = 21 kg). Each replicate exposure commenced with a volunteer opening a single 0.45 kg can of the product by pushing the seal tab into the can top and opening the multiple shaker holes. The volunteer then dusted 3 previously untreated dogs with the product, working the powder into the coat of each animal by hand. After completion of the task, the used can of insecticide was handed to the

study laboratory staff. To minimise cross contamination, individual areas were used for exposure and sampling. The floor was rinsed and towelled dry between replicates.

Detergent washes were then performed on the volunteer's hands, face and neck wipes were taken, and shirts, pants and whole body dosimeters were removed, sectioned, wrapped in aluminium foil and bagged. Samples were placed on dry ice and then stored frozen. Air pumps and tubing were cleaned after each replicate, and OVS sorbent tubes were capped, bagged and stored frozen. Volunteers were subsequently resuited and provided with a new air sampling tube before performing the second replicate.

To allow compensation for any effects of weathering on carbaryl, wash and wipe samples, OVS tubes and pieces of clothing and inner dosimeters were fortified with known amounts of the test chemical and exposed to the environment for 20 min under conditions similar to those during application. Blank control washing, wiping and clothing samples and OVS tubes were treated similarly. Handling and storage procedures were the same as those adopted for the samples from volunteers.

Carbaryl was extracted from facial wipes, dosimeters and external clothing sections with acetone and subjected to Florisil Bond Elut cleanup prior to analysis by HPLC. The same analytical method was used for handwash solutions following dichloromethane extraction and Florisil SPE cleanup if required. Carbaryl trapped in OVS sorbent tubes was extracted with acetonitrile for HPLC assay. The limit of quantitation for OVS tubes was 0.01 μg, and for all other samples was 1.0 μg.

Results:

Canine dusting appeared to offer considerable scope for operator exposure. Most volunteers held each dog against their body while applying the dust or otherwise made body contact with the animals, and in some cases caused a cloud of dust to rise into the air as they worked. Some dogs shed hair during treatment and dog hair was observed to adhere to the outer clothing of one volunteer.

The mean time spent applying the product to 3 dogs was 7 min (range = 5 – 13 min), during which a mean total of 65.3 g of dust was applied (equal to 3.5 g carbaryl, range = 0.65 – 10 g). Within the groups of 3 animals, the mean amount of carbaryl applied per dog was 1.2 g (range = 0.15 – 3.3 g/dog). When adjusted for bodyweight, the mean carbaryl dose per kg of 3 dogs was 57 mg/kg.

The average post-weathering recovery of carbaryl from fortified face/neck wipes, was 88%, and sample residue levels were adjusted accordingly. Over 90% recovery of carbaryl was achieved for hand washings, clothing and inner dosimeters and OVS sorbent tubes, and so no adjustments were made to field sample results from these matrices.

The highest residue levels of carbaryl were found on the external clothing, particularly the lower leg (110 – 37000 μg), upper leg (257 – 387000 μg) and lower arm (924 – 67900 μg). There was gross (up to 300-fold) inter-individual variation in the deposition of carbaryl on all parts of the shirt and pants. Total carbaryl residues on outer clothing are shown in the Table below.

Transfer of carbaryl across the outer clothing to the internal dosimeter was not extensive when averaged across the entire body, with a geometric mean value of 4.5% being obtained. However,

the lower arm was especially prone to exposure, with a maximum penetration rate of 28% being attained, although penetration exceeded 10% during only 13/40 replicate procedures. Exposures may have occurred under the cuff, which was sometimes observed to ride up the arm while treating dogs. Penetration through the pants was restricted to 0.1 – 6%.

The data suggested that although there was a tendency for the carbaryl levels on inner dosimeters to correlate positively with those on the outer clothing, the association was not consistent. There were numerous examples where a high deposition rate on the external clothing did not lead to extensive carbaryl residues on the inner dosimeter. Conversely, there were several examples where one or more individual sections of the inner dosimeter were more heavily contaminated than would be expected from carbaryl levels on the external clothing.

When gloves were not worn, there was extensive exposure via the hands, upon which carbaryl levels were 10-fold higher than those accumulating on the internal dosimeter (see Table) and accounted for 90% of total dermal residues. However, gloves reduced exposure to approximately 2% of the geometric mean carbaryl levels deposited on the unprotected hands. Again, there was gross variation in the amount of carbaryl detected, irrespective of whether gloves were worn.

Carbaryl levels found on the face and neck were low, being approximately 10% of those deposited on the inner dosimeter (see Table). The highest OVS sorbent tube residue was 71 μg, which would extrapolate to an inhalation exposure of 1027 μg, assuming a 29 L/min breathing volume.

When exposure from all sources (inhalation, hands, face, neck and inner dosimeter = skin under external clothing) was summed, the mean exposure to carbaryl was 1111 μg and 7986 μg, with and without gloves, respectively. When normalised for volunteer bodyweight and the amount of active constituent used, carbaryl exposure was 4.8 and 36 μg/kg bw/g applied, with and without gloves.

Exposure to Carbaryl by Volunteers Applying a 5.4% Powder Insecticide to Groups of 3 Dogs (Study 1517/10565)^

	Ungloved hands		**Gloved hands**	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
Application time (min)	7 ± 2	5.0 – 13	No separate data	-
Active constituent applied (g)	3.5 ± 1.8	0.65 – 10	No separate data	-
Total carbaryl residue on inner dosimeter (μg)	765 ± 3.4	63 – 13153	No separate data	-
Carbaryl on lower shirt sleeves (μg)	6727 ± 2.7	924 - 67900	No separate data	-
Carbaryl on lower trouser legs (μg)	1660 ± 5.3	110 - 37000	No separate data	-
Total carbaryl residue on outer clothing (μg)	16213 ± 2.8	1643 – 131190	No separate data	-
Carbaryl on hands (μg) N=20	6999 ± 1.6	3870 – 24600	124 ± 3.4	5.0 – 917
Carbaryl on face/neck (μg)	62 ± 2.6	8.9 – 320	No separate data	-

	Ungloved hands		Gloved hands	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
Total dermal exposure (μg)* N=20	7826	-	951	-
Est. carbaryl inhalation at 29 L/min breathing vol (μg)	160 ± 2.5	27 – 1027	No separate data	-
Total exposure (μg)*	7986	-	1111	-
Total exposure (μg active constituent/kg bw/g active constituent applied)	36	-	4.8	-

^N=40 replicates except where indicated otherwise. Arithmetic mean ± SD are given for application time and active constituent applied; all other results are expressed as geometric mean ± SD.

*Excludes carbaryl residues deposited on shirt and pants.

Conclusions:

During the application of a 5.4% powder insecticide product to 3 dogs, untrained volunteers were exposed to a mean of 1111 and 7986 μg carbaryl, respectively, when wearing or not wearing gloves. When adjusted for volunteer bodyweight and the amount of active constituent used, carbaryl exposure was 4.8 and 36 μg/kg bw/g applied, under the respective conditions.

Merricks DL (1997b) Carbaryl mixer/loader/applicator exposure study during application of RP-2 Liquid (21%), Sevin Ready To Use Insect Spray or Sevin 10 Dust to home garden vegetables. Study Nos: 1519 (Lab) and 10564 (Sponsor) Labs: Agrisearch Inc, Frederick, MD USA; Rhone-Poulenc Ag Company, Research Triangle Park, NC USA; and Morse Laboratories Inc, Sacramento, CA USA Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC USA Study duration: May 12 to December 15, 1997 Report date: December 15, 1997

QA: yes. GLP: US EPA 40 CFR Part 160 (1989) Test guideline: US EPA Subdivision U, Applicator Exposure Monitoring Series 231 and 232, Occupational and Residential Exposure Test Guidelines Group A, Application Exposure Test Guidelines

Study design:

This study assessed carbaryl inhalational and dermal exposure among a group of volunteers, under conditions intended to simulate use of 3 home garden insecticides in compliance with label directions.

The study group consisted of 70 male and non-pregnant female volunteers aged 19 – 72 y who had given informed consent prior to participation. None of the study group were professional spray applicators.

Three products were trialled:

- Sevin Liquid Brand Carbaryl Insecticide (Manufactured by Solaris Group/Aerofil Technology Inc, St Louis, MO USA, lot 20527/B1237), a 22.4% active constituent liquid formulation.

- Sevin Brand Ready-To-Use (Manufactured by Solaris Group/Contract Packaging Inc, Covington, GA USA, lots 705122 & 704500), a 0.11 – 0.14% active constituent liquid formulation.

- Sevin Brand Carbaryl Insecticide 10 Dust (Manufactured by Solaris Group/Aerofil Technology Inc, St Louis, MO USA, lot A21017A), a 9.8% active constituent powder formulation.

The non-active constituents present in Sevin Liquid Brand Carbaryl Insecticide are listed in Appendix IV. Excipients in the other products were not identified.

A total of 140 exposures was performed, according to the study design tabulated below:

Product	Application Equipment	Gloves	Replicates
Liquid (22.4%)	Hose-end sprayer	Yes	20
Liquid (22.4%)	Hose-end sprayer	No	20
Liquid (22.4%)	Hand-held pump sprayer	Yes	20
Liquid (22.4%)	Hand-held pump sprayer	No	20
Ready-To-Use (0.1%)	Pump bottle	Yes	20
Ready-To-Use (0.1%)	Pump bottle	No	20
Dust (9.8%)	Commercial duster	No	20

Each volunteer loaded (and where necessary, mixed) and applied one of the above products to two 6 m long rows of mature, vegetable-bearing tomatoes and a single 6 m long row of cucumbers, and then cleaned out the spray or dusting apparatus. (The Ready-To-Use pump bottle sprayer required no loading, mixing or clean up.) A second application was then performed using a different formulation or type of equipment. Throughout each procedure, volunteers wore cotton long sleeved shirts and long pants over a cotton whole body dosimeter. Breathing zone monitoring was performed using a personal air sampling pump operating at 2 L/min with an OVS sorbent tube containing XAD-2 resin. Air pumps were activated during the loading, mixing and clean up phases of the procedure, in addition to the application period.

When using a hose-end sprayer, the product was poured directly into the sprayer jar, which was then attached to the sprayer. The sprayer dial was set to 4 tsp [20 mL]/US gal [3.79 L] water and plants were sprayed to runoff at 275 kPa water pressure, delivered via a garden hose. Unused product was returned to the container and the spray equipment was rinsed with the hose.

Hand-held sprayers were filled with 2 US gal [7.6 L] of water, following which 8 tsp [40 mL] of the product was measured out, added, and mixed by agitation with the sprayer top closed. Further spraymix was prepared as necessary until the task was completed.

Sevin 10 Dust was added to a garden duster and pumped out onto the plants. The duster had an extended nozzle and discharged at about knee height if held vertically downwards. Any unused dust was emptied out and the duster was dismantled and rinsed with water.

Following application/clean up, detergent washes were performed on the volunteers' hands, face and neck wipes were taken, and shirts, pants and whole body dosimeters were removed, sectioned, wrapped in aluminium foil and bagged. Samples were placed on dry ice and then stored frozen. Air pumps and tubing were cleaned after each replicate, and OVS sorbent tubes were capped, bagged and stored frozen. Volunteers were subsequently resuited and provided with a new air sampling tube before performing any subsequent replicate.

To minimise cross contamination, the study area was designed to avoid walking through previously treated areas while conducting later replicates. Temperature, relative humidity, wind speed and wind direction were recorded during each replicate. To allow compensation for any effects of weathering on carbaryl, wash and wipe samples, OVS tubes and pieces of clothing and inner dosimeters were fortified with known amounts of the test chemical and exposed to the environment for 20 min under conditions similar to those during application. Blank control washing, wiping and clothing samples and OVS tubes were treated similarly. Handling and storage procedures were the same as those adopted for the samples from volunteers.

Carbaryl was extracted from facial wipes, dosimeters and external clothing sections with acetone and subjected to Florisil Bond Elut cleanup prior to analysis by HPLC. The same analytical method was used for handwash solutions following dichloromethane extraction and Florisil SPE cleanup if required. Carbaryl trapped in OVS sorbent tubes was extracted with acetonitrile for HPLC assay. The limit of quantitation for OVS tubes was 0.01 μg, and for all other samples was 1.0 μg.

Results:

Detailed observations were made of the volunteers as they performed the loading, mixing, spraying and clean up tasks. Depending on the product/application equipment, the entire procedure took an average of 18-23 min and consumed a mean amount of carbaryl that varied between 1 and 23 g:

Product	Application Equipment	Time (min)	Amount (g) of product applied	Amount (g) of carbaryl applied
Liquid (22.4%)	Hose-end sprayer	18	105	23.4
Liquid (22.4%)	Hand-held pump sprayer	23	33.6	7.5
Ready-To-Use (0.1%)	Pump bottle	18	826	1.1
Dust (9.8%)	Commercial duster	21	73.8	7.2

A number of the volunteers brushed against sprayed foliage, spilled or splashed themselves with spraymix, rinsate or dust, touched their faces, generated dust plumes or had to resolve problems with the equipment. During the exposure periods, the wind speed ranged from 0 – 11 km/h, the temperature lay between 20 and 33 °C, and humidity was 45 – 86%.

The average post-weathering recovery of carbaryl from fortified face/neck wipes, clothing and inner dosimeters was approximately 80%, and sample residue levels were adjusted accordingly. Some 91% recovery of carbaryl was achieved for hand washings and 98% for the active constituent in OVS sorbent tubes, and so no adjustments were made to field sample results from these matrices.

Sevin Liquid Brand Carbaryl Insecticide

Use of the hose-end sprayer resulted in approximately 3 times as much carbaryl being deposited on the external clothing and hands, compared with when hand-held pump sprayers were employed. However, the difference is attributable mainly to the larger amount of active constituent expended from the hose-end unit. For both sprayer types, the highest residue levels of carbaryl were found on the external clothing and ungloved hands. Indeed, if gloves were not worn, similar amounts of the active constituent were deposited on the hands as were detected on the total surface area of the shirt and pants (see Table). The most heavily contaminated parts of

the clothing were the lower and upper pants leg (up to 8121 and 1338 μg, respectively) and lower shirt sleeve (up to 400 μg with hand-held pumps and 3037 μg with hose-end units). There was extensive (70-fold) inter-individual variation in the total deposition of carbaryl on the external clothing and unprotected hands.

Gloves were highly effective at reducing manual contact with carbaryl. Carbaryl levels on the protected hand were less than 1% of those detected when gloves were not worn, irrespective of the type of sprayer used (see Table).

Transfer of carbaryl across most volunteers' outer clothing was not extensive. The majority of inner dosimeter samples contained no detectable residues. When the mean residue levels on the outer clothing and inner dosimeter are compared, overall penetration was approximately 1.2% with hand-held sprayers and 0.67% with hose-end sprayers. Localised penetration occurred most often across the lower shirtsleeves, but the highest amount of carbaryl detected in a single region was 180 μg, on the front torso of a volunteer who had used a hose-end sprayer. This individual had been observed to create splashback during clean up. Although there was a tendency for carbaryl levels on inner dosimeters to correlate positively with those on the outer clothing, the association was not consistent. There were instances where a high deposition rate on the external clothing failed to cause extensive carbaryl residues on the inner dosimeter. Conversely, there were a few examples where the inner dosimeter was more heavily contaminated than would be expected from carbaryl levels on the external clothing.

Carbaryl levels found on the face and neck were low or undetectable, using both sprayer types. Mean levels found on the face and neck were about 10% of those deposited on the inner dosimeter (see Table). Very little carbaryl was detected in the breathing zone air and the highest individual OVS tube residue was 0.03 μg, which would extrapolate to an inhalation exposure of only 0.38 μg, assuming a 29 L/min breathing volume.

Mean carbaryl exposure from all sources (inhalation, unprotected hands, face, neck and inner dosimeter = skin under external clothing) was 861 ug and 236 μg, using hose-end and hand-held pump sprayers, respectively. When adjusted for bodyweight and the amount of active constituent used, carbaryl exposure was 0.5 and 0.4 μg/kg bw/g applied, with the respective sprayer types. If gloves were worn, total exposure levels were reduced by factors of approximately130 and 40, with hose-end and hand pump sprayers, respectively.

Exposure to Carbaryl by Volunteers Mixing and Applying a 22.4% Liquid Insecticide to Vegetables (Study 1519/10564)

Spray apparatus	Hose-end sprayer		Hand pump sprayer	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
Application time (min)	4 ± 1	2 - 7	10 ± 3	3 – 17
Active constituent applied (g)	23 ± 9.5	5.0 – 49	7.5 ± 1.3	4.6 – 9.3
Total carbaryl residue on inner dosimeter (μg)	5.3 ± 2.6	3.0 – 184	4.3 ± 1.9	3.0 – 30
Total carbaryl residues on outer clothing (μg)	787 ± 3.4	31 – 8508	345 ± 2.8	69 – 4876
Carbaryl on hands (μg), **gloves worn** (N=20)	0.07 ± 3.35	<1.0 – 3.9	0.82 ± 2.8	<1.0 – 20
Carbaryl on hands (μg), **unprotected** (N=20)	830 ± 3.1	63 – 4440	242 ± 2.4	51 – 2100

Carbaryl on face/neck (μg)	0.61 ± 2.52	<1.0 – 58	0.54 ± 1.6	<1.0 – 9.8
Total dermal exposure (μg)*, **gloves worn** (N=20)	6.9	-	5.8	-
Total dermal exposure (μg)*, **hands unprotected** (N=20)	861	-	236	-
Est. carbaryl inhalation at 29 L/min breathing vol (μg)**	0.09 ± 1.5	0.07 – 0.25	0.14 ± 2.0	0.07 – 0.49
Total exposure , **gloves worn** (μg active constituent/kg bw/g active constituent applied) (N = 20)	0.004	-	0.011	-
Total exposure , **hands unprotected** (μg active constituent/kg bw/g active constituent applied) (N=20)	0.49	-	0.44	-

^ N=40 except where stated otherwise. Arithmetic mean ± SD are given for application time and active constituent applied; all other results are expressed as geometric mean ± SD.

*Not presented by study author; calculated by multiplying μg exposure/g active constituent by amount of active constituent applied. Excludes carbaryl residues deposited on shirt and pants.

**For calculation purposes, ½ limit of quantitation (0.005 μg) was used when the residue was not detected.

Sevin Brand Ready-To-Use

This product was approximately 2 orders of magnitude less concentrated than the others tested. There was a commensurate reduction in the amount of carbaryl deposited on the skin and clothing (see Table). When exposure from all sources was summed, the mean dermal exposure to carbaryl was 96 μg. No carbaryl residues were detected on the gloved hands, and gloves effected a 95% reduction in total dermal exposure to the active constituent. The extent of inhalation exposure was negligible.

When adjusted for bodyweight and the amount of active constituent used, carbaryl exposure was 1.2 μg/kg bw/g applied if gloves were not worn, and 0.06 μg/kg bw/g if applied with gloves. It is noteworthy that volunteers' exposure to carbaryl from the ready-to-use product was greater per gram of the active constituent applied, than during use of the more concentrated Sevin Liquid Brand. This finding is unexpected, given that the ready-to-use product required no dilution, transfer to other spray equipment or clean up procedure, which should have reduced the potential for exposure.

Exposure to Carbaryl by Volunteers Applying a 0.1% Liquid Insecticide to Vegetables (Study 1519/10564)^

Spray apparatus	**Ready-to-use pump bottle sprayer**	
	Mean ± SD	**Range**
Application time (min)	9 ± 2	6 – 13
Active constituent applied (g)	1.05 ± 0.10	0.89 – 1.26
Total carbaryl residue on inner dosimeter (μg)	3.9 ± 1.7	3.0 – 62
Total carbaryl residues on outer clothing (μg)	37 ± 2.6	4.2 – 173
Carbaryl on hands (μg), **gloves worn** (N=20)	Not Detected	-
Carbaryl on hands (μg), **unprotected** (N=20)	83 ± 3.5	3.7 – 654
Carbaryl on face/neck (μg)	0.54 ± 1.65	1.0 – 11.9
Total dermal exposure (μg)*, **gloves worn** (N=20)	5.2	-
Total dermal exposure (μg)*, **hands unprotected** (N=20)	96	-
Estimated carbaryl inhalation at 29 L/min breathing vol (μg)**	0.15 ± 2.26	0.07 – 0.88

Total exposure , **gloves worn** (μg active constituent/kg bw/g active constituent applied) (N = 20)	0.06	-
Total exposure , **hands unprotected** (μg active constituent /kg bw/g active constituent applied) (N=20)	1.2	-

^ N=40 except where stated otherwise. Arithmetic mean ± SD are given for application time and active constituent applied; all other results are expressed as geometric mean ± SD.

*Not presented by study author; calculated by multiplying μg exposure/g active constituent by amount of active constituent applied. Excludes carbaryl residues deposited on shirt and pants.

**For calculation purposes, ½ limit of quantitation (0.005 μg) was used when the residue was not detected.

Sevin Brand Carbaryl Insecticide 10 Dust

Vegetable dusting generated mean total dermal carbaryl residue levels that were similar to those caused by applying liquid product via hose-end sprayer without gloves.

The highest mean residue levels of carbaryl were found on the external clothing. The distribution pattern was more even than caused by spray application, with a relatively smaller difference between the most heavily contaminated areas (lower arm, 91 – 1815 μg, and front torso, 24 – 1737 μg) and the least contaminated (rear torso, 11 – 411 μg). Total carbaryl residues on outer clothing are shown in Table.

Transfer of carbaryl across the outer clothing to the internal dosimeter was moderate when averaged across the entire body, with a geometric mean value of 4.9% being obtained. The highest localised penetration rates occurred across the lower arm and rear torso, with mean values of 7.1% and 8.5%, respectively.

As with the liquid products applied without gloves, carbaryl dust residues on the hands accounted for over 90% of total dermal exposure, and carbaryl levels on the hands were more than 10-fold higher than those accumulating on the internal dosimeter (see Table).

Carbaryl levels found on the face and neck were 10% of those deposited on the inner dosimeter (see Table). The highest OVS sorbent tube residue was 4 μg, which would extrapolate to an inhalation exposure of 58 μg, assuming a 29 L/min breathing volume. Although the face and inhaled air accounted for less than 2% of total exposure, it is noteworthy that volunteers applying the dust received approximately 10 times more exposure on the face, and 100 times more inhalation exposure compared to those using the hose-end and hand-held pump sprayers.

Mean carbaryl exposure from all sources (inhalation, hands, face, neck and inner dosimeter = skin under external clothing) was 1181 μg. When normalised for volunteer bodyweight and the amount of active constituent used, carbaryl exposure was 2.1 μg/kg bw/g applied.

Exposure to Carbaryl by Volunteers Applying a 9.8% Dust Insecticide to Vegetables (Study 1519/10564)^

Apparatus	**Duster**	
	Mean ± SD	**Range**
Application time (min)	7.0 ± 1.0	4.0 – 9.0
Active constituent applied (g)	7.2 ± 4.8	1.2 – 20
Total carbaryl residue on inner dosimeter (μg)	66 ± 1.9	22 – 171
Total carbaryl residues on outer clothing (μg)	1269 ±1.8	249 – 3260
Carbaryl on hands (μg), unprotected	863 ± 2.6	228 – 13300

Carbaryl on face/neck (μg)	6.6 ± 2.6	<1.2 – 34
Total dermal exposure (μg)*, hands unprotected	1166	-
Est. carbaryl inhalation at 29 L/min breathing vol (μg)**	11 ± 4.37	0.07 – 58
Total exposure , hands unprotected (μg active constituent/kg bw/g active constituent applied) (N=20)	2.1	-

^ N=20. Arithmetic mean ± SD are given for application time and active constituent applied; all other results are expressed as geometric mean ± SD.

*Not presented by study author; calculated by multiplying μg exposure/g active constituent by amount of active constituent applied. Excludes carbaryl residues deposited on shirt and pants.

**For calculation purposes, ½ limit of quantitation (0.005 μg) was used when the residue was not detected.

Conclusions:

When untrained volunteers applied a 22.4% liquid product to vegetables, the mean exposure to carbaryl was 861 μg and 236 μg, using hose-end and hand-held pump sprayers, respectively. When adjusted for bodyweight and the amount of active constituent used, carbaryl exposure was 0.5 and 0.4 μg/kg bw/g applied, with the respective sprayer types. If gloves were worn, total exposure rates were reduced to 0.004 and 0.011μg/kg bw/g applied, with hose-end and hand pump sprayers, respectively.

Use of a 0.1% ready-to-use liquid, which was applied directly from its pump bottle package, resulted in a mean exposure to carbaryl of 96 μg. Gloves effected a 95% reduction in dermal exposure to the active constituent. When adjusted for bodyweight and the amount of active constituent used, carbaryl exposure was 1.2 μg/kg bw/g applied if gloves were not worn, and 0.06 μg/kg bw/g if applied with gloves.

When the volunteers treated vegetables with a 9.8% dust product, the mean exposure to carbaryl was 1181 μg. When normalised for volunteer bodyweight and the amount of active constituent used, carbaryl exposure was 2.1 μg/kg bw/g applied.

It is noteworthy that exposure from carbaryl dust was approximately 4-fold higher than observed when liquid concentrate product was applied to the same area of vegetables by hose-end and hand-held pump sprayers, and double the exposure rate when applying the ready-to-use product.

Merricks DL (1998) Carbaryl mixer/loader/applicator exposure study during application of RP-2 Liquid (21%) to fruit trees and ornamental plants. Study Nos: 1518 (Lab) and 10564 (Sponsor) Labs: Agrisearch Inc, Frederick, MD USA; Rhone-Poulenc Ag Company, Research Triangle Park, NC USA; and Morse Laboratories Inc, Sacramento, CA USA Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC USA Study duration: April 7, 1997 to January 23, 1998 Report date: January 23, 1998

QA: yes. GLP: US EPA 40 CFR Part 160 (1989) Test guideline: US EPA Subdivision U, Applicator Exposure Monitoring Series 231 and 232, Occupational and Residential Exposure Test Guidelines Group A, Application Exposure Test Guidelines

Study design:

This study assessed carbaryl inhalational and dermal exposure among a group of volunteers, under conditions intended to simulate use of a home garden insecticide in compliance with label directions.

The study group consisted of 20 male and non-pregnant female volunteers aged 18 – 66 y who had given informed consent prior to participation. The volunteers' mean weight was 80.7 kg and mean height was 1.75 m. None of the study group were professional spray applicators.

The product used was Sevin Liquid Brand Carbaryl Insecticide (Manufactured by Solaris Group/Aerofil Technology Inc, St Louis, MO USA, lot 20527/B1237), a 22.4% active constituent liquid formulation registered by US EPA for use on fruit and nut trees, vegetables, ornamentals, flowers and shrubs. The non-active constituents present in the product are listed in Appendix III.

A total of 40 replicate exposures was performed. Twenty exposures were by hose-end sprayer, while the remainder employed a hand-held pump sprayer. Each volunteer conducted one replicate with each type of equipment. Throughout the procedure, volunteers wore cotton long sleeved shirts and long pants over a cotton whole body dosimeter. No hat or gloves were worn. Breathing zone monitoring was performed using a personal air sampling pump operating at 2 L/min with an OVS sorbent tube containing XAD-2 resin. Pumps were activated during the loading, mixing and clean up phases of the procedure, in addition to the application period.

The procedure commenced by opening the product bottle. When using a hose-end sprayer, the product was poured directly into the sprayer jar, which was then attached to the sprayer. The sprayer dial was set to 4 tsp [20 mL]/US gal [3.79 L] water and 2 citrus trees 2-3 m tall and 2 ornamental plants 1 – 1.5 m tall were sprayed to runoff using a 3 US gal/min water flow, delivered via a garden hose. Unused product was returned to the container and the spray equipment was rinsed with the hose.

Detergent washes were then performed on the hands, face and neck wipes were taken, and shirts, pants and whole body dosimeters were removed, sectioned, wrapped in aluminium foil and bagged. Samples were placed on dry ice and then stored frozen. Air pumps and tubing were cleaned after each replicate, and OVS sorbent tubes were capped, bagged and stored frozen.

Volunteers were subsequently resuited and provided with a new air sampling tube before performing the pump sprayer replicate. A 2 US gal [7.6 L] volume of water was placed in the sprayer, following which 8 tsp [40 mL] of the product were measured out, added, and mixed by agitation with the sprayer top closed. Further spraymix was prepared as necessary. Two citrus trees and 2 ornamentals were sprayed as before, residual spraymix was discarded, and the spray equipment was rinsed by hose. The volunteers' outer clothing, inner dosimeter, face and hands were then treated as described previously.

To minimise cross contamination, the study area was designed to avoid walking through previously treated areas while conducting later replicates. Temperature, relative humidity, wind speed and wind direction were recorded during each replicate. To allow compensation for any effects of weathering on carbaryl, wash and wipe samples, OVS tubes and pieces of clothing and inner dosimeters were fortified with known amounts of the test chemical and exposed to the environment for 20 min under conditions similar to those during application. Blank control washing, wiping and clothing samples and OVS tubes were treated similarly. Handling and storage procedures were the same as those adopted for the samples from volunteers. A storage stability study was also undertaken over a 32-wk period with all sample matrices except OVS tubes.

Carbaryl was extracted from facial wipes, dosimeters and external clothing sections with acetone and subjected to Florisil Bond Elut cleanup prior to analysis by HPLC. The same analytical method was used for handwash solutions following dichloromethane extraction and Florisil SPE cleanup if required. Carbaryl trapped in OVS sorbent tubes was extracted with acetonitrile for HPLC assay. The limit of quantitation for OVS tubes was 0.01 μg, and for all other samples was 1.0 μg.

Results:

Detailed observations were made of the volunteers as they performed the loading, mixing, spraying and clean up tasks. The entire procedure took an average of 13 min (range 8 – 18 min) when using the hose-end sprayer and 18 min (range 12 – 21 min) when using the hand-held sprayer. A number of the volunteers brushed against sprayed foliage or had to resolve problems when loading or cleaning the spray equipment. During the exposure periods, the wind speed ranged from 0 – 16 km/h, the temperature lay between 14 and 27 °C, and humidity was 40 – 95%.

Application by hose-end sprayer was accomplished in approximately one third of the time required to treat the same number of trees by hand-held pump sprayer. However, approximately twice as much carbaryl was expended when using the hose-end unit than with the hand-held pump sprayer (see Table). The mean elapsed time from commencement to completion of the load/mix/spray/clean up procedure was 13 min (range = 8 – 18 min) with a hose-end sprayer and 18 min (range = 13 – 22 min) with a hand-held pump.

The average post-weathering recovery of carbaryl from fortified face/neck wipes, clothing and inner dosimeters was approximately 80%, and sample residue levels were adjusted accordingly. Approximately 90% recovery of carbaryl was achieved for hand washings and OVS sorbent tubes, and so no adjustments were made to field sample results from these matrices.

For both sprayer types, the highest geometric mean residue levels of carbaryl were found on the external clothing, particularly the leg (168 – 493 μg) and lower arm (66 – 100 μg). The lowest residues were present on the rear torso (8.5 – 20 μg) and upper arm (11 – 18 μg), followed by the front torso (30 – 39 μg). There was gross (60 to 900-fold) inter-individual variation in the deposition of carbaryl on the leg and lower arm. The study authors believed that much of the variability was caused by splashes during clean up, rather than residues deposited when spraying. Total carbaryl residues on outer clothing are shown in the Table below.

Transfer of carbaryl across the outer clothing was not extensive. Most of the inner dosimeter samples contained no detectable residues. The highest penetration rate was 4.5%, from the lower arm to the inner dosimeter. Penetration through the pants was restricted to 0.1 – 4%, possibly because the pants were made of thicker material than the shirt, and had less tendency to become wetted during spraying or clean up. The data suggested that although there was a tendency for the carbaryl levels on inner dosimeters to correlate positively with those on the outer clothing, the association was not consistent. There were numerous examples where a high deposition rate on the external clothing did not lead to extensive carbaryl residues on the inner dosimeter. Conversely, there were several examples where the inner dosimeter was more heavily contaminated than would be expected from carbaryl levels on the external clothing.

By far the largest source of exposure was the hands, upon which carbaryl levels were similar to those accumulating on the entire external clothing (see Table). Residues were slightly higher

following use of the hose-end unit than the hand-held pump sprayer. But if the greater active constituent use from the hose-end unit is taken into consideration, this type of apparatus was associated with less carbaryl deposition per g applied. Again, there was gross variation in the amount of carbaryl detected. The highest residue of 13200 µg was attained following use of the hose-end sprayer, approximately 20 times greater than the mean from either type of apparatus, and over 100-fold greater than the lowest hand residue levels.

Carbaryl levels found on the face and neck were low or undetectable, using both sprayer types. Mean levels found on the face and neck were about 10% of those deposited on the inner dosimeter (see Table). Very little carbaryl was detected in the breathing zone air and the highest OVS tube residue was 0.03 µg, which would extrapolate to an inhalation exposure of only 0.38 µg, assuming a 29 L/min breathing volume.

When exposure from all sources (inhalation, hands, face, neck and inner dosimeter = skin under external clothing) was summed, the mean exposure to carbaryl was 743 ug and 524 µg, when using hose-end and hand-held pump sprayers, respectively. When normalised for bodyweight and the amount of active constituent used, carbaryl exposure was 0.6 and 0.8 µg/kg bw/g applied, with the respective sprayer types.

Exposure to Carbaryl by Volunteers Mixing and Applying a 22.4% Liquid Insecticide to 4 Trees (Study 1518/10564)^

Spray apparatus	Hose-end sprayer		Hand pump sprayer	
	Mean ± SD	Range	Mean ± SD	Range
Application time (min)	4.0 ± 2.0	2.0 – 7.0	11 ± 2.0	8.0 – 14
Active constituent applied (g)	17 ± 9.4	9.1 – 41	8.0 ± 1.0	5.8 – 9.3
Total carbaryl residue on inner dosimeter (µg)	6.1 ± 2.2	3.0 – 27	7.6 ± 2.0	3.0 – 34
Total carbaryl residues on outer clothing (µg)	685 ± 4.0	59 – 8629	1074 ± 3.0	28 – 5277
Carbaryl on hands (µg)	737 ± 4.0	82 – 13200	516 ± 2 .0	139 – 3080
Carbaryl on face/neck (µg)	0.6 ± 1.8	<1.0 – 3.6	0.7 ±2.4	<1.0 – 20
Total dermal exposure (µg)*	743	-	524	-
Est. carbaryl inhalation at 29 L/min breathing vol (µg)**	0.09 ± 1.51	0.07 – 0.29	0.12 ± 1.96	0.07 – 0.38
Total exposure (µg active constituent/kg bw/g active constituent applied)	0.62	-	0.83	-

^ N=20. Arithmetic mean ± SD are given for application time and active constituent applied; all other results are expressed as geometric mean ± SD.
*Excludes carbaryl residues deposited on shirt and pants.
**For calculation purposes, ½ limit of quantitation (0.005 µg) was used when the residue was not detected.

Conclusions:

Under simulated home garden conditions involving the application of a 22.4% liquid insecticide product to 2 large and 2 small trees, untrained volunteers were exposed to a mean of 743 and 524 µg carbaryl when using hose-end and hand-held spray apparatus, respectively. Greater than 99% of exposure was via the ungloved hands. When normalised for bodyweight and the amount of

active constituent used, carbaryl exposure was 0.6 and 0.8 μg/kg bw/g applied, with the respective sprayer types.

1.4.10 Assessment of Systemic Uptake of Carbaryl from Use of HG/HV Products

Under normal conditions of use, systemic uptake of carbaryl from HG/HV products would occur via inhalation and dermal contact during application, and by ingestion of treated home grown fruit and vegetables. This assessment will therefore examine both aspects of householder exposure, and also take into account additional dietary intake from commodities purchased commercially. Inhalational and dermal absorption factors can be estimated from available data, combined with the above exposure measurements, and be incorporated into a systemic uptake model for users of carbaryl sprays and dusts.

Models of user exposure

The exposure studies by Merricks (1997a, 1997b, 1998) are fairly representative of product application under home garden/veterinary conditions by untrained users, and yielded detailed data on the potential for inhalation exposure, together with the amount and distribution of carbaryl deposited on the skin and clothing of product users. The spray/dust apparatus employed by the applicators was similar to equipment available to Australian gardeners, while the amount of treatment work done was realistic for persons who own pets, have fruit trees, or grow vegetables in domestic premises. While not all the tested American products had the same active constituent concentration as their Australian counterparts, preparation methods and dilution rates were described comprehensively. Data from Merricks' studies can therefore be applied to Australian registered pet or plant dusts, wettable powders and liquid spray products, provided due allowance is made for any inter-regional differences in use pattern. However, the data cannot be used for other Australian product classes including flea collars, veterinary ear drops, carpet treatments and baits, as their use pattern and/or composition differ markedly from the American products that were tested.

The only shortfall in study design was that no data were obtained on the amount of carbaryl deposited on the product users' footwear. Given the significant deposition of carbaryl on the lower leg in most of the tested scenarios, it may be assumed that deposition on the feet will also have occurred. Thus, total carbaryl exposure is likely to have been underestimated, but to an unknown extent. Ground level application of sprays or dusts and use of pet dusts would probably offer most scope for carbaryl deposition on the feet. Based on the demonstrably low penetration rate of carbaryl across cotton clothing, deposition on the foot skin is expected to be negligible if shoes or boots are worn. Nevertheless, outside a controlled experimental setting, some users will not wear enclosed footwear, especially when no such requirement is included in the label safety directions.

Although inhalation and dermal deposition were characterised, Merricks made no attempt to measure the total systemic doses of carbaryl that were absorbed by the volunteers. His studies do not, in themselves, enable any judgement to be made as to any toxicological hazard that would arise from the exposure scenarios that were investigated. Given that the aim of this assessment is to compare the systemic carbaryl doses achieved by product users with toxicological benchmarks such as the ADI and ARfD, it is therefore necessary to estimate the amount of carbaryl that would be absorbed.

Factors influencing systemic carbaryl dose

Clothing/personal protective equipment:

In all the submitted user exposure studies, the subjects wore long pants and long sleeved shirts buttoned to the wrist. There was heavy deposition of carbaryl on the lower sleeves and trouser leg, which would otherwise have reached the skin if briefer clothing had been worn. Many Australian users of carbaryl products will also wear long pants and sleeves, but others will not, especially during the warmer months of the year when the products are most likely to be applied. Furthermore, current Australian label safety directions for carbaryl based products do not advise users to wear long pants or sleeves or equivalent clothing that would reduce carbaryl deposition on the skin. Fortunately, the user exposure studies measured carbaryl residues on specific regions of the external clothing including the lower sleeves and pants legs, so enabling calculation of the additional exposure that would have occurred with short sleeves and pants. To allow for variation in user clothing, two sets of dose estimates will be prepared, one for persons wearing long clothing and the other for persons wearing shorts and a T-shirt or equivalent.

The only personal protective equipment worn by the American study subjects were disposable latex gloves. Most (but not all) of the tested scenarios were repeated twice, one replicate being run with gloves and the other, without. Exposure estimates for Australian products will therefore be calculated from "gloves on" data if gloves are required by the current label safety directions, and from "gloves off" data if users are not directed to wear them.

Bodyweight:

The mean weight of subjects participating in the American exposure studies was approximately 80 kg. This figure is considered to be excessive for the Australian population, especially females and adolescents. Systemic doses have therefore been calculated assuming the WHO average human bodyweight of 64 kg.

Concentration of active constituent in the product and spray mixture:

The influence of carbaryl concentration in the product/spray mixture on dermal and inhalation exposure was not examined in the American applicator studies. In cases where the carbaryl concentration in Australian dust products differs from the equivalent American test product, estimated exposure levels will be adjusted proportionally (ie, if extrapolating from a 10% dust to a 5% dust, measured carbaryl deposition levels would be halved).

With wettable powders and liquids that require dilution before use, the situation is more complicated. The American exposure studies utilised a 22% liquid concentrate but no wettable powders, whereas a variety of liquid concentrates (6-50% active constituent) and wettable powders (8-80% active constituent) are available on the Australian HG market. It is not known how much of the study subjects' total exposure occurred during the individual measuring/mixing, spraying and clean up phases of the procedure. It is therefore unclear whether the active constituent concentration in the product would exert a greater influence on user exposure than its concentration in the spray mixture. The reviewer is also unable to predict the extent of exposure from measuring out and mixing a liquid, compared with a wettable powder.

For the purposes of systemic dose estimation, it has been assumed that spray mixture concentration will be a determinant, and so estimated exposure levels will be adjusted proportionally when extrapolating from data on American products. The absence of suitable data prevents adjustment for active constituent concentration or whether the product is in liquid or powder form.

Apparatus used in application:

While the American exposure studies revealed a possible spray equipment related influence on carbaryl exposure per weight active constituent applied, similar dust and spray equipment is used in Australia and America. There is no perceived requirement to make any adjustment for the type of equipment used in Australia.

Amount of carbaryl inhaled:

The amount of carbaryl inhaled during use of Australian products is likely to be equivalent to the American findings, once adjusted for differences in c active constituent concentration in dusts or spray mixtures. Although only limited experimental data are available on inhalation uptake of carbaryl, the chemical appears to be well absorbed across the lungs in studies evaluated by the IPCS [in EHC 153 (WHO 1994)]. About 50% of the dose was absorbed by anaesthetised rats within 2.6 min following administration of a carbaryl solution via the pulmonary route. In a separate experiment, approximately 75% of 14C-labelled carbaryl was retained by rats when inhaled as a vapour for 1 h.

Therefore, for human user systemic uptake modelling purposes it has been assumed that there would be complete absorption of inhaled carbaryl.

Amount of carbaryl absorbed across the skin:

A similar extent of carbaryl skin deposition is anticipated from Australian products as their US equivalents, subject to adjustment for clothing, personal protective equipment and active constituent concentration. However, estimating the extent of dermal absorption of carbaryl is problematic despite the subject having been studied using a variety of *in vitro* and *in vivo* test systems, including human volunteers. A summary of results is tabulated below.

Dermal absorption of carbaryl in various test systems

***In vitro* studies**				
Test System	**Mixture Applied**	**Extent of Absorption of Applied Dose**	**Remarks**	**Reference**
Rat skin	Carbaryl in acetone	1% over 7.5 h into Earles' MEM receptor medium 2.7% over 7.5 h into 1:1 ethanol:water receptor medium	Study authors reported that skin cell metabolic activity was maintained in presence of Earles' MEM but not ethanol/water	MacPherson *et al.* (1991)

Flow-through diffusion study, porcine skin SLS=sodium lauryl sulfate DMSO =dimethyl sulfoxide Krebs-Ringer bicarb/dextrose/ bovine serum albumin receptor medium	Carbaryl in: 40% acetone 40% acetone/1% SLS 40% DMSO 40% DMSO/1% SLS	1% over 1 h* 3% over 2 h* 9.5% over 8 h 0.3% over 1 h* 2.2% over 2 h* 17% over 8 h 0.1% over 1 h* 0.3% over 2 h* 2.9% over 8 h 0.2 % over 1 h* 0.6% over 2 h* 13% over 8 h *values estimated by evaluator	With acetone as solvent, peak carbaryl uptake was 3-4% of dose/h, attained at 2-3 h and subsequently declining. With DMSO as solvent, rate of carbaryl uptake was 0.1-0.2% of dose/h during initial 60 min but increased with increasing duration of dermal contact.	Baynes & Riviere (1998)
Flow-through diffusion study, latex gloves	40% acetone 40% DMSO	82% over 8 h 39% over 8 h		Baynes & Riviere (1998)

***In vivo* studies**				
Test Species	**Mixture Applied**	**Extent of Absorption of Applied Dose**	**Remarks**	**Reference**
Rat	Carbaryl in acetone	5.8% over 1 h 58% over 168 h	Radioactivity measured in excreta, tissues	Knaak *et al.* (1984)
Rat	Carbaryl in acetone, 0.15, 0.54 or 2.68 μmol/cm^2 for 72 h	30% of low dose 20% of mid dose 4% of high dose (all over 72 h)	Radioactivity measured in excreta, tissues	Shah *et al.* (1987)
Rat	440 or 4400 μg XLR plus (44% active constituent) in 1% CMC for 24 h	2% over 30 min 25% over 24 h	Radioactivity measured in excreta, tissues	Cheng (1995)
Rat	800 μg Sevin 80S (80% active constituent) in 1% CMC for 24 h	0.7% over 30 min 14% over 24 h	Radioactivity measured in excreta, tissues	Cheng (1994)
Human	Carbaryl in acetone applied to jaw angle for 24 h	4.4% over 4 h 16% over 8 h 70% over 120 h	Estimated from urinary excretion	Maibach *et al.* (1971)
Human	Carbaryl in acetone applied to forearm for 24 h	0.2% over 4 h 5% over 8 h 74% over 120 h	Estimated from urinary excretion	Maibach *et al.* (1971)
Human	Carbaryl in acetone applied to forearm for 24 h	2%/h over 0-4 h with 3.5 h lag time 35% over 8 h 77% over 120 h	Estimated from above data* by regular constrained deconvolution	Fisher *et al.* (1985)

*The same experimental results were reported twice, firstly by Maibach *et al.* (1971) and then Feldmann & Maibach (1974). In the latter paper, which was used as data source by Fisher *et al.*, carbaryl absorption across the forearm is given as 0.005% of the dose per h during the 4 h post application (ie, 0.02% over 0 – 4 h). This figure is 10-fold less than stated by Maibach *et al.* (1971); the data sets are otherwise in agreement. It is therefore possible that Fisher *et al.* have underestimated carbaryl absorption during the initial 4 h of exposure, or that their posited 3.5 h latency period is artefactual.

Carbaryl is moderately well absorbed across the skin, especially following prolonged contact. Taken at face value, the *in vitro* studies suggest a penetration rate of between 0.1 and 2% per h, while the *in vivo* studies indicate rates ranging from 0.05 to 5.8% per h.

However, dermal absorption appears subject to variation mediated by the experimental methods. The *in vitro* study of Baynes and Riviere (1998) demonstrates that acetone vehicle enhances carbaryl uptake across porcine skin, compared with DMSO (3% *vs.* 0.3% of the dose over 2 h, respectively). Acetone has been employed as a vehicle in most of the available *in vivo* studies, including those on humans (Maibach *et al.*, 1971). Studies performed with acetone vehicle are therefore of dubious relevance to carbaryl absorption from dusts, spray mixture and even liquid concentrates, as acetone is not present in Australian products for which composition data are available. Paradoxically, although Maibach *et al.* 's choice of vehicle may have enhanced dermal penetration of carbaryl (at least immediately post-application), the extent of absorption is likely to have been underestimated over the 4 h time span, due to reliance on urinary excretion of the chemical. This would not account for carbaryl that had been absorbed but had yet to be excreted. Their study also reveals markedly greater carbaryl absorption from the jaw skin as compared to the forearm skin.

The most well documented and relevant *in vivo* studies (Cheng, 1994 & 1995) have been previously submitted by Rhone-Poulenc and were evaluated by OCS in 1998. Dermal absorption of radiolabelled carbaryl across rat skin from two products was found to be moderately extensive but saturable. The most extensive absorption occurred with Carbaryl XLR Plus (an aqueous concentrate containing 43.9% carbaryl), applied in 1% aqueous carboxymethylcellulose (CMC) vehicle. Some 2% of a 440 or 4400 μg/animal dermal dose was absorbed over 30 min, rising to 25% over 24 h. When the applied dose was increased to 44000 μg/animal, dermal absorption reached only 3.2% of administered radioactivity over 24 h. With Sevin 80S (80.1% carbaryl wettable powder), applied at 800, 8000 or 40000 μg/animal in 1% CMC), dermal absorption at the lowest dose amounted to 0.7% over 30 min and 14% at 24 h. When 8000 or 44000 μg/animal were applied, dermal absorption attained only 1.2% of administered radioactivity over a 24-h period.

The two Cheng studies are a particularly suitable basis for estimating systemic uptake of carbaryl by HG/HV product users. Both test compounds were commercial products similar to those sold in Australia, an aqueous vehicle was used to administer them, and radioactivity was quantified in all body tissues and excreta. Although the authors showed that the proportion of carbaryl absorbed decreases with increasing dose, it is difficult to apply this aspect of their findings to the user uptake model. In a real life scenario, carbaryl deposition will be heavier on some areas of a user's skin than others, and so the percentage uptake will vary across different regions of the body.

The available data set does not provide appropriate information to enable judgement as to whether dermal uptake from a liquid carbaryl preparation would be more or less extensive than from a dust or powder. Although Cheng (1994, 1995) tested a liquid and a powder in the same experimental system, both test formulations were mixed with 1% aqueous CMC before application and so were not an exact match to either spray mixture or to the undiluted products.

It cannot be assumed that dermal absorption from dusts or powders would always be half as extensive as from liquids, so the dermal absorption factor chosen for the user uptake model is therefore 2% of the applied dose in 30 min (ie 4%/h), the maximum rate of systemic uptake reported by Cheng.

It is noteworthy that the US EPA has estimated a 12.7% dermal absorption factor for carbaryl by reference to these same studies (R Zendzian, personal communication, June 2000). However, the EPA factor is intended for estimating occupational exposure and is therefore considered too high for the HG or HV product scenario, which would involve a shorter duration of exposure.

Duration of contact with the skin:

Given that most subjects in the US exposure studies spent 15 – 30 min performing the whole task from loading to clean up, it is assumed that Australian HG/HV product users would also usually be exposed to carbaryl for up to 30 min, during which 2% of the dermal dose would be absorbed.

In a real life situation, though, some users may not wash their exposed skin immediately after application or clean up, even when directed to do so by the product label. While this would have no effect on the extent of inhalation uptake, failure to wash immediately after use would prolong dermal contact with carbaryl and increase the amount absorbed across the skin. The systemic uptake model will therefore incorporate a second set of estimates based on a 2 h dermal contact time, during which 8% of the dermal dose would be absorbed.

Dermal transfer factors

Following product application, it is likely that the user or other members of the household make contact with treated pets, turf or other surfaces. Where necessary, US EPA procedures (1997, 1999) will be used to estimate the amount of carbaryl that would be dislodged from treated animals/substrates and transferred to the skin.

User exposure model for HV 5% carbaryl pet dusts/powders - dog treatment

Reference data: Merricks (1997a): Application of 5% dust to groups of 3 dogs, without gloves. Exposure estimates in this model will be reduced to one third of measured values to yield estimates for treatment of 1 dog, weighing approximately 20 kg.

Application equipment: Shaker dispenser.

Adjustment for active constituent concentration in product: Nil. The American test product and equivalent Australian registered products contain 50 g/kg carbaryl.

Personal protective equipment: Nil. None required by current label safety directions.

Dust treatment of 1 dog: typical user exposure and dose with long sleeved shirt and long pants, based on mean data

One third of measured geometric mean user exposure, µg		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal	2609	52	208
Inhalational	53	53	53
TOTAL	2662	105	261
Bw-adjusted systemic dose (µg/kg)		**1.6**	**4**
Systemic dose as % of ARfD		16	40
Systemic dose as % of ADI		21	50

Note: Extrapolating from geometric mean data obtained by Merricks (1997a), total exposure would be reduced from 2662 to approximately 370 µg if gloves were worn in addition to long pants and a long sleeved shirt.

Dust treatment of 1 dog: "worst case" user exposure and dose with long sleeved shirt and long pants, based on top of range data

One third of measured top of range user exposure, µg		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal	12691	254	1015
Inhalational	342	342	342
TOTAL	13033	596	1357
Bw-adjusted systemic dose (µg/kg)		**9**	**21**
Systemic dose as % of ARfD		90	210
Systemic dose as % of ADI		117	265

Note: Extrapolating from top of range data obtained by Merricks (1997a), total exposure would be reduced from 13033 to approximately 5139 µg if gloves were worn in addition to long pants and a long sleeved shirt. However, the worst case inhalation dose alone (342/64 = 5.3 µg/kg) amounts to half the ARfD and 67% of the ADI.

Dust treatment of 1 dog: typical user exposure and dose with short sleeved shirt and short pants, based on mean data

One third of measured geometric mean user exposure, µg		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	2242	45	179
Dermal: lower legs	553	11	44
Dermal: rest of skin	2609	52	208
Inhalational	53	53	53

TOTAL	5458	161	485
Bw-adjusted systemic dose (µg/kg)		**2.5**	**8**
Systemic dose as % of ARfD		25	80
Systemic dose as % of ADI		32	95

Dust treatment of 1 dog: "worst case" user exposure and dose with short sleeved shirt and short pants, based on top of range data

One third of measured top of range user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal: lower arms	22633	453	1811
Dermal: lower legs	12333	247	987
Dermal: rest of skin	12691	254	1015
Inhalational	342	342	342
TOTAL	48000	1295	4154
Bw-adjusted systemic dose (µg/kg)		**20**	**65**
Systemic dose as % of ARfD		200	650
Systemic dose as % of ADI		253	812

It is also necessary to consider potential subsequent exposure when making contact with carbaryl residues in the fur when handling or grooming the treated animal. The US EPA (1997) currently assumes that 20% of pesticide residues are retained on pet fur as dislodgeable residue, and that 10% of the dislodgeable residues are transferred to the pet handler. The mean carbaryl dose applied in the reference study was 1.2 g/dog. If 2% of this dose became transferred onto the handler's skin, the handler would be exposed to 24 mg (24000 µg) carbaryl. While inhalation exposure may also occur, there is no basis upon which it may be estimated. Inhalation exposure has therefore been omitted from the calculation.

Estimated dermal exposure and dose from handling a dog already treated with carbaryl dust

Estimated handler exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal	24 000	480	1920
Bw-adjusted systemic dose (µg/kg)		**7.5**	**30**
Systemic dose as % of ARfD		75	300
Systemic dose as % of ADI		94	375

An initial appraisal of the results would suggest a possible toxicological hazard if exposure extended over 2 h, with the ARfD and ADI being exceeded by 3- and 3.8-fold, respectively. However, such high systemic doses are unlikely to be attained in practice, given that the current US EPA method yields a dermal exposure estimate for handling (24 000 µg) that is an order of magnitude higher than the geometric mean exposure observed during application of the pesticide (2600 µg).

Conclusions:

The toxicological hazard from treating one 20 kg dog is considered **unacceptable**, because the measured inhalation and dermal exposure to carbaryl is sufficient to deliver systemic doses that exceed the ARfD and ADI under reasonably foreseeable conditions of use. Even if dermal exposure could be eliminated, inhalation exposure alone would be sufficient to deliver a systemic dose equivalent to half the ARfD per dog treated. Although many householders own small dogs or cats, which would probably require less carbaryl to be applied during treatment, ownership of two or more medium to large sized dogs is common. Some product users may therefore be at risk of even heavier exposure than has been estimated.

Consideration has been given to revising the label safety directions for HV 5% pet dusts/powders. To confer adequate protection, users would need to wear cotton overalls buttoned to the neck or wrist (or equivalent clothing), together with rubber gloves and a disposable dust mask. However, this level of protective clothing and equipment would be perceived as incompatible with products intended for treating companion animals. A high rate of user non-compliance is considered likely.

Given that carbaryl based flea collars and shampoos would cause markedly less user exposure than dusts, especially by inhalation, it is considered prudent to recommend the withdrawal of home veterinary dusts/powders containing carbaryl.

User exposure model for HV 5% carbaryl pet dusts/powders – bird treatment

Reference data: Merricks (1997a): Application of 5% dust to groups of 3 dogs, without gloves.

Application equipment: Puffer pack.

Adjustment for active constituent concentration in product: Nil. The American test product and Australian registered bird dusts contain 50 g/kg carbaryl.

Personal protective equipment: Nil. None required by current label safety directions.

Exposure scenario: Carbaryl dusts are applied to caged birds for control of lice and mites. The available product label instructs users to squeeze the container quickly and firmly, directing the resultant dust cloud towards the bird. The cage floor and perches are also to be dusted "liberally" and the dust left in contact for 1 h, after which it is to be removed.

Personal experience of Office of Chemical Safety staff suggests that there is considerable potential for user exposure to the active constituent. Some owners hold their birds while dusting them to ensure treatment under the wings and tail feathers; approximately 1 g of dust is used to treat each small bird (finches, budgerigars) while more would be required for larger birds (parrots, pigeons). It is not uncommon for aviculturists to keep up to 50 small birds or 30 pigeons on domestic premises, and simultaneous disinfestation of the entire aviary would consume 50 g of the product or more. Thus, an amateur aviculturist could apply about 2.5 g of carbaryl to the birds and aviary, and be reexposed when removing contaminated litter and dust from the cage. Furthermore, at least some aviculturists routinely add carbaryl dust to nesting boxes, one author specifically recommending a product formulated as a European Wasp killer for this purpose.

The extent of human exposure when applying carbaryl dust to birds is unknown. In the absence of specific data generated during bird disinfestation, it will be assumed that the exposure pattern and rate is similar to that measured by Merricks (1997a) in volunteers applying 5% carbaryl dust to dogs. In Merricks' study, users applied an average of 1.2 g carbaryl/dog, and so a bird owner expending 2.5 g active constituent would be exposed to approximately double the amount of carbaryl compared with the estimated exposure from dusting 1 dog.

Treatment of aviary: estimated typical user exposure and dose with long sleeved shirt and long pants

Estimated geometric mean user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal	5220	104	416
Inhalational	110	110	110
TOTAL	5330	214	526
Bw-adjusted systemic dose (µg/kg)		**3.3**	**8.2**
Systemic dose as % of ARfD		33	82
Systemic dose as % of ADI		42	103

Note: Extrapolating from geometric mean data obtained by Merricks (1997a), total exposure would be reduced from 5330 to approximately 740 µg if gloves were worn in addition to long pants and a long sleeved shirt.

Treatment of aviary: "worst case" user exposure and dose with long sleeved shirt and long pants

Estimated top of range user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal	25400	508	2032
Inhalational	680	680	680
TOTAL	26080	1188	2714
Bw-adjusted systemic dose (µg/kg)		**19**	**42**
Systemic dose as % of ARfD		190	420
Systemic dose as % of ADI		233	525

Note: Extrapolating from top of range data obtained by Merricks (1997a), total exposure would be reduced from 26080 to approximately 9750 µg if gloves were worn in addition to long pants and a long sleeved shirt. However, the worst case inhalation dose alone (680/64 = 10.6 µg/kg) amounts to the ARfD and 133% of the ADI.

Treatment of aviary: estimated typical user exposure and dose with short sleeved shirt and short pants

Estimated geometric mean user exposure, µg		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	4480	90	360
Dermal: lower legs	1110	22	88
Dermal: rest of skin	5220	104	416
Inhalational	110	110	110
TOTAL	10900	326	974
Bw-adjusted systemic dose (µg/kg)		**5.1**	**15**
Systemic dose as % of ARfD		51	150
Systemic dose as % of ADI		64	188

Treatment of aviary: estimated "worst case" user exposure and dose with short sleeved shirt and short pants

Estimated top of range user exposure, µg		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	45270	905	3620
Dermal: lower legs	24670	493	1972
Dermal: rest of skin	25400	508	2032
Inhalational	680	680	680
TOTAL	96000	2586	8304
Bw-adjusted systemic dose (µg/kg)		**40**	**130**
Systemic dose as % of ARfD		400	1300
1.4.10.1 Systemic dose as % of ADI		500	1625

Conclusions:

The toxicological hazard from treating a large aviary is considered **unacceptable**, because the estimated inhalation and dermal exposure to carbaryl would be sufficient to deliver systemic doses exceeding the ARfD and ADI under reasonably foreseeable conditions of use. Even if dermal exposure could be eliminated, inhalation exposure alone would be sufficient to deliver a systemic dose equivalent to the ARfD.

Although many bird owners would keep only 1 or 2 birds in a small cage, and would not absorb toxicologically significant amounts of carbaryl when applying treatment even under worst case conditions, ownership of up to 50 birds is not uncommon. It is probably unfeasible to impose

label restrictions on the number of birds that may be treated simultaneously. Two further possible courses of regulatory action have therefore been considered.

The first of these is to revise the label safety directions for HV 5% avian dusts/powders. To confer adequate protection, users would need to wear cotton overalls buttoned to the neck or wrist (or equivalent clothing), together with rubber gloves and a disposable dust mask. However, this level of protective clothing and equipment may be perceived as unrealistic, especially by persons owning a few birds, and a high rate of non-compliance is likely.

User exposure model for HV 1% ear drops

Reference data: Nil

Application equipment: Not known.

Adjustment for active constituent concentration in product: Nil

Personal protective equipment: Nil

Exposure scenario: The two registered ear drop products are supplied in 20 or 25 mL bottles. Users are directed to apply "several drops" to the affected animal's ear canals twice daily for 14 d. Under normal circumstances, there would be no exposure of the person applying the drops, and carbaryl is not expected to be transferred from within the ear canal to the animal's fur. The "worst case" that could be reasonably anticipated, would involve spillage of 1 mL of the liquid formulation on the exposed skin of the hand or forearm. At present, no safety directions have been set for this class of product. The label of one ear drop product warns users not to inhale vapour and to avoid contact with eyes and skin, but there is no instruction to wash hands after use or if the liquid is spilled onto the skin. The product may therefore remain in contact with the skin for some time following an accidental splash or spill.

A 1 mL volume of the ear drop preparations, contains 10 mg carbaryl (ie 10,000 μg). Other constituents present in the products for which compositional data are available, are not expected to enhance dermal penetration of carbaryl within the maximum envisaged exposure time of 2 h.

Ear drop: user exposure and dose following accidental spill

Estimated user exposure, μg		**Estimated μg uptake over 30 min (2% dermal penetration)**	**Estimated μg uptake over 2 h (8% dermal penetration)**
Dermal	10 000	200	800
Inhalational	-	-	-
TOTAL	10 000	200	800
Bw-adjusted systemic dose (μg/kg)		**3.1**	**13**
Systemic dose as % of ARfD		31	125
Systemic dose as % of ADI		39	157

NOTE: The above data are for a single exposure only, and may underestimate potential daily dose as two daily applications are required.

Conclusions:

Ear drop preparations are considered **not to pose a significant hazard** to the user, as there would be no exposure to carbaryl under normal conditions of use. While an accidental spill could deliver sufficient carbaryl to exceed the ARfD and ADI, removal of the chemical within 30 min would restrict systemic absorption to below these doses. Any risk to the user can be satisfactorily mitigated by setting label safety directions that include the statements 210 164 ("Avoid contact with skin") and 351 ("Wash hands after use").

User exposure model for 1% HV shampoos and foams

Reference data: No relevant human exposure studies available.

Personal protective equipment: Nil. No safety directions have been set for veterinary shampoos.

Exposure pattern: According to the product labels, a 20 mL volume or greater is to be applied. Based on the personal experience of dog owners within the Office of Chemical Safety, up to 15 min would be required to lather, massage and rinse the animal. A 20 mL volume of shampoo contains 200 mg (200,000 μg) carbaryl. The US EPA (1997) assumes that 10% of the active constituent applied by shampoo would come into contact with the pet groomer. Hence, an estimated 20 000 μg carbaryl would come into contact with the user's hand and forearm skin for 15 min, assuming that the rinsing process would result in complete removal of carbaryl from the user.

1% carbaryl shampoo: user exposure and dose following application of to one animal

Estimated user exposure, μg		**Estimated μg uptake over 15 min (1% dermal penetration)**
Dermal	20 000	200
Inhalational	-	-
TOTAL	20 000	200
Bw-adjusted systemic dose (μg/kg)		**3.2**
Systemic dose as % of ARfD		32
Systemic dose as % of ADI		40

Although carbaryl residues would remain on the pet's skin and coat after washing, no data are available on the amount that would be present or would subsequently transfer onto humans. The US EPA (1997, 1999) currently assumes that 20% of pesticide residues on pet fur are dislodgeable, and that 10% of dislodged residue is transferred to a person handling the treated pet. Applying the overall 2% factor to the estimated amount of carbaryl applied (200 000 μg) would yield an estimated dermal exposure of 4 000 μg to a handler.

1% carbaryl shampoo: estimated exposure and dose from handling a treated animal

Estimated handler exposure, μg		**Estimated μg uptake over 30 min (2% dermal penetration)**	**Estimated μg uptake over 2 h (8% dermal penetration)**
Dermal	4 000	80	320
Bw-adjusted systemic dose (μg/kg)		**1.26**	**5.0**

Systemic dose as % of ARfD	12.6	50
Systemic dose as % of ADI	16	64

Dermal exposure to 4000 μg carbaryl would therefore not result in a toxicologically significant systemic dose. In practice, some of the 200 000 μg carbaryl applied would be rinsed off, and so even a 4000 μg exposure is unlikely to be attained.

Conclusions:

The above exposure model suggests that use of 10 g/L pet shampoos **would not cause a significant hazard** to persons applying them to one or two animals, or handling treated animals. However, consistent with the principle that pesticide exposure should be reduced as low as reasonably achievable, and to assure protection of persons treating three or more animals, an entry should be established in the FAISD Handbook, directing product users to wear rubber gloves when opening the container and using the product (279 280 283 290 312).

User exposure model for 5% HG dusts applied to external garden areas and vegetables

Reference data: Merricks (1997b): Exposure study during application of 10% dust to home garden vegetables, without gloves.

Application equipment: Merricks' study involved application with duster, whereas at least some Australian products are supplied in a shaker dispenser. In the absence of any data on use of shaker dispensers on vegetables, exposure from shaker dispensers will be assumed to be equivalent to dusters.

Adjustment for active constituent concentration in product: The garden dust exposure trials undertaken by Merricks (1997b) were performed with a 10% product. To obtain the exposure/systemic dose levels caused by 5% dusts registered in Australia, the exposure levels from 10% carbaryl dust will be halved.

Personal protective equipment: Nil. None required by current label safety directions.

5% dusts: typical user exposure and dose with long sleeved shirt and long pants, based on mean data

Measured geometric mean user exposure, μg, adjusted downwards by 50%		**Estimated μg uptake over 30 min (2% dermal penetration)**	**Estimated μg uptake over 2 h (8% dermal penetration)**
Dermal	583	12	47
Inhalational	6	6	6
TOTAL	589	18	53
Bw-adjusted systemic dose (μg/kg)		**0.28**	**0.82**
Systemic dose as % of ARfD		2.8	8.2
Systemic dose as % of ADI		3.5	11

5% dusts: "worst case" user exposure and dose with long sleeved shirt and long pants, based on top of range data

Measured top of range user exposure, µg, adjusted downwards by 50%		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal	6753	135	540
Inhalational	58	58	58
TOTAL	6811	193	598
Bw-adjusted systemic dose (µg/kg)		**3.02**	**9.3**
Systemic dose as % of ARfD		30	93
Systemic dose as % of ADI		38	117

Note: Merricks (1997b) did not measure operator exposure with gloves.

5% dusts: typical user exposure and dose with short sleeved shirt and short pants, based on mean data

Measured geometric mean user exposure, µg, adjusted downwards by 50%		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	141	2.8	11
Dermal: lower legs	133	2.7	11
Dermal: rest of skin	583	12	47
Inhalational	6	6	6
TOTAL	722	20	63
Bw-adjusted systemic dose (µg/kg)		**0.32**	**0.99**
Systemic dose as % of ARfD		3.2	9.9
Systemic dose as % of ADI		4.0	13

5% dusts: "worst case" user exposure and dose with short sleeved shirt and short pants, based on top of range data

Measured top of range user exposure, µg, adjusted downwards by 50%		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	908	18	73
Dermal: lower legs	641	13	51
Dermal: rest of skin	6753	135	540
Inhalational	58	58	58
TOTAL	8360	224	722
Bw-adjusted systemic dose (µg/kg)		**3.5**	**11**
Systemic dose as % of ARfD		35	113
Systemic dose as % of ADI		44	142

Conclusions:

There is potential for persons applying 5% carbaryl dusts to external garden areas and vegetables to absorb systemic doses of carbaryl that exceed the ARfD and ADI under worst-case conditions. However, the field data shows that the potential for exposure to carbaryl from dusting vegetables is markedly less than when dusting dogs, especially with regard to inhalation exposure. **Provided persons using 5% garden dusts wear gloves and appropriate clothing, they should not be exposed to toxicologically significant doses** of carbaryl.

It is recommended that the FAISD Handbook entry for 50 g/kg (or less) dusts be modified by inclusion of the statements 279 280 283 290 292b 312 ("When opening the container and using the product wear cotton overalls buttoned to the neck and wrist [or equivalent clothing] and rubber gloves").

User exposure model for 2% HG dusts applied to vegetables, flowers and ornamentals

Reference data: Merricks (1997b): Exposure study during application of 10% dust to home garden vegetables, without gloves.

Application equipment: Merricks' study involved application with duster, whereas at least some Australian products are supplied in a shaker dispenser. In the absence of any data on use of shaker dispensers on vegetables, exposure from shaker dispensers will be assumed to be equivalent to dusters.

Adjustment for active constituent concentration in product: As in the previous section, the exposure studies were performed using a 10% product. To obtain the exposure/systemic dose levels caused by 2% dust, the exposure levels from 10% carbaryl dust will be reduced by 5-fold.

Personal protective equipment: Nil. None required by current label safety directions.

2% dusts: typical user exposure and dose with long sleeved shirt and long pants, based on mean data

Measured geometric mean user exposure, μg, adjusted downwards by 80%		**Estimated μg uptake over 30 min (2% dermal penetration)**	**Estimated μg uptake over 2 h (8% dermal penetration)**
Dermal	233	4.7	19
Inhalational	2.4	2.4	2.4
TOTAL	235	7.1	21
Bw-adjusted systemic dose (μg/kg)		**0.11**	**0.33**
Systemic dose as % of ARfD		1.1	3.3
Systemic dose as % of ADI		1.4	4.1

2% dusts: "worst case" user exposure and dose with long sleeved shirt and long pants, based on top of range data

Measured top of range user exposure, µg, adjusted downwards by 80%		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal	2701	54	216
Inhalational	23	23	23
TOTAL	2724	77	239
Bw-adjusted systemic dose (µg/kg)		**1.2**	**3.7**
Systemic dose as % of ARfD		8.3	37
Systemic dose as % of ADI		17	47

2% dusts: typical user exposure and dose with short sleeved shirt and short pants, based on mean data

Measured geometric mean user exposure, µg, adjusted downwards by 80%		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	56	2.82	11.3
Dermal: lower legs	53	2.66	10.6
Dermal: rest of skin	233	11.7	46.6
Inhalational	2.4	2.4	2.4
TOTAL	289	8.2	25
Bw-adjusted systemic dose (µg/kg)		**0.13**	**0.40**
Systemic dose as % of ARfD		1.3	4.0
Systemic dose as % of ADI		1.5	5.0

2% dusts: "worst case" user exposure and dose with short sleeved shirt and short pants, based on top of range data

Measured top of range user exposure, µg, adjusted downwards by 80%		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	363	7.3	29
Dermal: lower legs	256	5.1	21
Dermal: rest of skin	2701	54	216
Inhalational	23	23	23
TOTAL	3344	90	289
Bw-adjusted systemic dose (µg/kg)		**1.4**	**4.5**
Systemic dose as % of ARfD		14	45
Systemic dose as % of ADI		18	57

Conclusions:

There is low potential for persons applying 2% carbaryl dusts to external garden areas and vegetables to absorb systemic doses of carbaryl that exceed the ADI or ARfD. **Any such possibility will be obviated if users wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and rubber gloves**.

User exposure model for 5% HG insecticidal carpet deodoriser dust

The single product in this category is sold in a shaker dispenser of 375 g pack size. The dust is to be sprinkled lightly over carpets, rugs and pet bedding, where it is allowed to lie for at least 1 h before removal by vacuum cleaner. Treatments are recommended at 14-d intervals or as required.

No personal protective equipment is recommended in the FAISD Handbook for dusts containing 50 g/kg or less of carbaryl. The product label warns users to avoid contact with eyes and skin, to not inhale dust, and to wash hands after use, as recommended by the FAISD Handbook. There is a further warning to avoid contact with the treated carpet before vacuuming.

User exposure during application of the carpet deodoriser has not been modelled, but may be similar to levels achieved when applying 5% dusts to garden vegetables. However, these data do not address the potential for systemic exposure of persons (in particular infants) making contact with treated carpet. Carbaryl levels remaining on treated carpet have not been measured and cannot be estimated, being subject to variation with the amount dispensed, the type of material treated and the efficiency of the vacuum cleaning process. Further exposure to carbaryl when reentering the treated area, vacuuming and disposing of vacuum cleaner dust may also occur. It is therefore not possible to quantify the extent of exposure of household occupants from the 5% carpet insecticide/deodoriser, but the potential for exposure is considered to be high.

In this context, it is noteworthy that the IPCS EHC Review on carbaryl cites a report in which a 5% surface spray of carbaryl was applied for insect control in homes. One week after treatment, inhibition of plasma ChE activity was found in 48 out of 63 residents.

Conclusions:

In the absence of sufficient relevant data that would assure the safety of household occupants using this type of product, **it is advisable to recommend the withdrawal of carbaryl dusts or powders intended for indoor carpet treatment**. It would be advisable also to prohibit the use of carbaryl for broadcast treatment within domestic premises.

User exposure model for 1.8% HG cricket/grasshopper bait

The single product in this category is sold in 1 and 12.5 kg packs with dispenser tray. The bait consists of shaved grain flakes, which are to be scattered in the garden or placed in the dispenser tray. Although no safety directions for this type of product appear in the FAISD Handbook, the product label directs users to avoid contact with the skin and eyes and avoid breathing dust, and to wash exposed parts of the body after use and before eating, drinking or smoking.

No information exists which would allow estimation of user exposure to carbaryl while dispensing the product. However, compared with use of carbaryl dusts or powders, there would appear to be a lower potential for the bait flakes to be inhaled or deposited dermally, as the bait is expected to have a significantly larger mean particle size. The exterior use pattern of the product would also tend to limit the extent of exposure.

Conclusions:

No significant toxicological hazard is anticipated from use of this product. An entry covering baits containing 18 g/kg or less of carbaryl should be established in the FAISD Handbook, including statements 210 164 ("Avoid contact with skin") and 351 ("Wash hands after use").

User exposure model for HG wettable powders applied to fruit trees and ornamental plants

Reference data: No studies have been provided covering the use of WP products. The most relevant data were obtained by Merricks (1998) during application of a 22% carbaryl liquid to trees by hand pump sprayer, without gloves. Given that the potential for user exposure from spray mixture would be the same once the powder or liquid concentrate had been mixed with water, data from the study on the liquid product are applied here for estimating user exposure to WPs.

Application equipment: Based on the directions on available product labels, it is expected that users will employ a hand-held pump sprayer.

Adjustment for active constituent concentration in product: WPs registered in Australia contain between 80 and 800 g/kg carbaryl, and are diluted to 1 – 1.25 g active constituent/L water for application to fruit and vegetables. The effect of active constituent concentration in the undiluted product was not examined in the submitted studies, and so there is no "off the shelf" adjustment factor that can be incorporated into the exposure model. Intuitively, a more concentrated product might be expected to result in higher user exposure levels when measuring out and diluting the powder concentrate. However, this assumption is not necessarily justified. A critical determinant of user exposure is the amount of active constituent handled and applied, which in turn depends on the number and size of plants requiring treatment. Thus, irrespective of whether a home gardener was using an 80 or 800 g/L product, for a given crop size the amount of carbaryl applied should remain constant. Users would simply handle a proportionally lower volume of the more concentrated powder. Once the spray mixture has been prepared, the original product concentration would have little influence on user exposure during spraying or clean up. Consequently, no adjustment will be made for carbaryl concentration in the products.

Adjustment for active constituent concentration in spray mixture: Australian carbaryl WPs are diluted to 1 – 1.25 g active constituent/L water for application to fruit and vegetables. Sevin Liquid Brand Carbaryl Insecticide, the American SC product used in the reference study, was applied at the rate of 40 mL product/7.6 L water, ie 1.18 g active constituent/L. Therefore, no adjustment has been made for active constituent concentration in the spray mixture.

Personal protective equipment: Current recommended safety directions for WP products (all strengths) specify elbow-length PVC gloves when preparing the spray, but not when using the product. In practice, manufacturers differ in their approach, with some labels further advising that gloves should be worn during use, while others do not. In Merricks' study, user exposure was measured after the final clean up, and there are no separate data on exposure occurring

during the individual preparation, application and clean up phases. It is therefore not possible to allow for the use of gloves during only part of the sequence. Furthermore, while most users would be careful to avoid exposure during spray mix preparation, they may show less caution when handling the diluted spray mixture, especially in the absence of label directions (on some products) to wear gloves. The following exposure model is therefore based on data obtained when gloves were not worn by the study subjects.

Carbaryl WPs: tree treatment - estimated typical user exposure and dose with long sleeved shirt and long pants, based on mean data

Geometric mean user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal	524	11	42
Inhalational	0.12	0.12	0.12
TOTAL	524	11	42
Bw-adjusted systemic dose (µg/kg)		**0.17**	**0.66**
Systemic dose as % of ARfD		1.7	6.6
Systemic dose as % of ADI		2.2	8.3

Carbaryl WPs: tree treatment - estimated "worst case" user exposure and dose with long sleeved shirt and long pants, based on top of range data

Top of range user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal	3134	63	252
Inhalational	0.38	0.38	0.38
TOTAL	3134	63	252
Bw-adjusted systemic dose (µg/kg)		**0.98**	**3.9**
Systemic dose as % of ARfD		9.8	39
Systemic dose as % of ADI		12	49

Carbaryl WPs: tree treatment - estimated typical user exposure and dose with short sleeved shirt and short pants, based on mean data

Geometric mean user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal: lower arms	66	1.3	5.3
Dermal: lower legs	264	5.3	21
Dermal: rest of skin	3134	63	252
Inhalational	0.38	0.38	0.38
TOTAL	3464	70	279
Bw-adjusted systemic dose (µg/kg)		**1.1**	**4.4**
Systemic dose as % of ARfD		11	44
Systemic dose as % of ADI		14	55

Carbaryl WPs: tree treatment - estimated "worst case" user exposure and dose with short sleeved shirt and short pants, based on top of range data

Top of range user exposure, μg		Estimated μg uptake over 30 min (2% dermal penetration)	Estimated μg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	906	18	72
Dermal: lower legs	1297	26	104
Dermal: rest of skin	3134	63	252
Inhalational	0.38	0.38	0.38
TOTAL	5337	107	428
Bw-adjusted systemic dose (μg/kg)		**1.7**	**6.7**
Systemic dose as % of ARfD		17	67
Systemic dose as % of ADI		21	84

Conclusions

There is potential for persons not wearing long pants and sleeves while applying carbaryl wettable powders to trees and ornamentals, to absorb systemic doses of carbaryl that approach the ADI. However, the USA field data shows negligible potential for inhalation exposure to carbaryl when spraying trees, and that gloves alone are capable of reducing user exposure by 40-fold or more. **Provided persons using WPs wear gloves and appropriate clothing, they should not be exposed to toxicologically significant doses of carbaryl.** Although the current Safety Directions recommend that gloves be worn when preparing the spray, they do not do so when spraying. Furthermore, users are not directed to wear long pants and sleeves. It is therefore recommended that the FAISD Handbook entry for wettable powders be modified by inclusion of the statements 279 280 281 282 290 292b 312 ("When opening the container, preparing spray and using the prepared spray wear cotton overalls buttoned to the neck and wrist [or equivalent clothing] and rubber gloves").

[NOTE: Safety directions for carbaryl wettable powders will be reviewed following the forthcoming APVMA review of carbaryl residues in food.]

User exposure model for HG wettable powders applied to vegetables

Reference data: No studies have been provided that cover the use of WP products. The nearest relevant data were obtained by Merricks (1997b) during application of a 22% carbaryl liquid to vegetables by hand pump sprayer, without gloves. Given that the potential for user exposure from spray mixture would be the same once the powder or liquid concentrate had been diluted, data from the study on the liquid product are applied here for estimating user exposure to WPs.

Application equipment: Based on the directions on available product labels, it is expected that users will employ a hand-held pump sprayer.

Adjustment for active constituent concentration in product: WPs registered in Australia contain between 80 and 800 g/kg carbaryl, and are diluted to 1 – 1.25 g active constituent/L water for application to fruit and vegetables. The effect of active constituent concentration in the

undiluted product was not examined in the submitted studies, and so there is no "off the shelf" adjustment factor that can be incorporated into the exposure model. Intuitively, a more concentrated product might be expected to result in higher user exposure levels when measuring out and diluting the powder concentrate. However, this assumption is not necessarily justified. A critical determinant of user exposure is the amount of active constituent handled and applied, which in turn depends on the number and size of plants requiring treatment. Thus, irrespective of whether a home gardener was using an 80 or 800 g/L product, for a given crop size the amount of carbaryl applied would remain constant. Users would simply handle a proportionally lower volume of the more concentrated powder. Once the spray mixture has been prepared, the original product concentration would have little influence on user exposure during spraying or clean up. Consequently, no adjustment will be made for carbaryl concentration in the products.

Adjustment for active constituent concentration in spray mixture: Australian carbaryl WPs are diluted to 1 – 1.25 g active constituent/L water for application to fruit and vegetables. Sevin Liquid Brand Carbaryl Insecticide, the American SC product used in the reference study, was applied at the rate of 40 mL product/7.6 L water, ie 1.18 g active constituent/L. No adjustment is therefore required for active constituent concentration in the spray mixture.

Personal protective equipment: Current recommended safety directions for WP products (all strengths) specify elbow-length PVC gloves when preparing the spray, but not when using the product. In practice, manufacturers differ in their approach, with some labels further advising that gloves should be worn during use, while others do not. In Merricks' study, user exposure was measured after the final clean up, and there are no separate data on exposure occurring during the individual preparation, application and clean up phases. It is therefore not possible to allow for the use of gloves during only part of the sequence. Furthermore, while most users would be careful to avoid exposure during spray mix preparation, they may show less caution when handling the diluted spray mixture, especially in the absence of label directions (on some products) to wear gloves. The following exposure model is therefore based on data obtained when gloves were not worn by the study subjects.

Carbaryl WPs: vegetable treatment - typical user exposure and dose with long sleeved shirt and long pants, based on mean data

Geometric mean user exposure, μg		**Estimated μg uptake over 30 min (2% dermal penetration)**	**Estimated μg uptake over 2 h (8% dermal penetration)**
Dermal	236	4.7	19
Inhalational	0.14	0.14	0.14
TOTAL	236	4.9	19
Bw-adjusted systemic dose (μg/kg)		**0.08**	**0.30**
Systemic dose as % of ARfD		0.8	3
Systemic dose as % of ADI		1	3.5

Carbaryl WPs: vegetable treatment - "worst case" user exposure and dose with long sleeved shirt and long pants, based on top of range data

1.4.10.1.1 Top of range user exposure, µg		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal	2140	43	171
Inhalational	0.49	0.49	0.49
TOTAL	2140	43	172
Bw-adjusted systemic dose (µg/kg)		**0.67**	**2.7**
Systemic dose as % of ARfD		6.7	27
Systemic dose as % of ADI		9	34

Carbaryl WPs: vegetable treatment - typical user exposure and dose with short sleeved shirt and short pants, based on mean data

Geometric mean user exposure, µg		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	8	0.16	0.64
Dermal: lower legs	191	3.8	15
Dermal: rest of skin	236	4.7	19
Inhalational	0.14	0.14	0.14
TOTAL	435	8.8	35
Bw-adjusted systemic dose (µg/kg)		**0.14**	**0.55**
Systemic dose as % of ARfD		1.4	5.5
Systemic dose as % of ADI		1.8	7.0

Carbaryl WPs: vegetable treatment - "worst case" user exposure and dose with short sleeved shirt and short pants, based on top of range data

Top of range user exposure, µg		Estimated µg uptake over 30 min (2% dermal penetration)	Estimated µg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	400	8	32
Dermal: lower legs	3449	69	276
Dermal: rest of skin	2140	43	171
Inhalational	0.49	0.49	0.49
TOTAL	5989	120	479
Bw-adjusted systemic dose (µg/kg)		**1.9**	**7.5**
Systemic dose as % of ARfD		19	75
Systemic dose as % of ADI		24	94

Conclusions

The potential user exposure when applying WP sprays to garden vegetables is similar to that occurring while spraying trees. In both scenarios, it would be possible to absorb systemic doses of carbaryl that approach the ADI if long pants and sleeves were not worn. **Revision of the FAISD Handbook entry to recommend gloves and overalls (or equivalent) are worn while using the spray, is considered sufficient to cover use on vegetables.**

[NOTE: Safety directions for carbaryl wettable powders will be reviewed following the forthcoming APVMA review of carbaryl residues in food.]

User exposure model for 60 g/l "hose-on" lawn grub insecticide

Reference data: The most relevant data were obtained by Merricks (1997b) during application of a 22% carbaryl liquid to vegetables by hose-end sprayer, without gloves. It is anticipated that turf application by spray would result in a similar pattern of dermal exposure to treatment of vegetables.

Application equipment: Product is sold in a 2L click-on hose-end spray bottle.

Adjustment for differences between the American study product and Australian product: The American product employed in Merricks' study contained 22% carbaryl. It was decanted into a hose-end sprayer and applied at 20 mL product/3.8L water, equivalent to 4.4 g active constituent/3.8L = 1.2 g/L carbaryl. An average of 23 g carbaryl was applied (range = 5 – 49 g), after which any remaining concentrate was emptied from the sprayer prior to cleaning the apparatus.

By comparison, the Australian product is more dilute at 6% carbaryl and is not decanted from its container, which connects directly to the hose via a click-on fitting. Under mains pressure, water mixes automatically with the concentrate at the correct ratio, but the final active constituent concentration in the spray mixture is unknown. The apparatus is disposed of when depleted and cleanup appears unnecessary. The reservoir holds 2L of concentrate (ie 120 g carbaryl) which is sufficient to treat 400 m^2 turf. An average Australian dwelling would have approximately 200 m^2 lawn area, which would require application of about 60 g carbaryl.

Since Merricks' study did not provide separate measurements of user exposure during the individual preparation, application and cleanup phases, it is not possible to adjust for the fact that the Australian product requires no preparation or cleanup. Furthermore, in the absence of data on active constituent concentration in the Australian product's effluxive spray, it is not possible to compensate for any differences that may exist in the final carbaryl level delivered by the two products.

Consequently, the only adjustment that can be made to the American exposure data is to multiply the measured exposure levels by a factor of 2.5, compensating for the increased amount of carbaryl that would be applied to turf using the Australian product (60 *vs.* 23 g).

Personal protective equipment: When the exposure model was prepared, the product label complied with the safety directions recommended in the FAISD Handbook for WP, LD and SC carbaryl products of all strengths, stating that elbow-length PVC gloves should be worn *when*

preparing spray. Users were not directed to wear gloves during the spraying process itself, which is when the majority of exposure would occur with this product. Hence, the exposure model assumes that gloves are not worn.

Exposure scenarios: Two exposure scenarios are envisaged. The first of these is exposure of a product user, assuming they are not wearing gloves and that the treatment area is 200 m^2. The second is householder exposure from contact with treated turf.

Lawn grub killer: typical user exposure and dose with long sleeved shirt and long pants, based on mean data

Estimated user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal	2153	43	172
Inhalational	0.23	0.23	0.23
TOTAL	2153	43	172
Bw-adjusted systemic dose (µg/kg)		**0.67**	**2.69**
Systemic dose as % of ARfD		6.7	27
Systemic dose as % of ADI		8.5	34

Note: Extrapolating from geometric mean data obtained by Merricks (1997b), total exposure would be reduced to approximately 18 µg if gloves were worn in addition to long pants and a long sleeved shirt.

Lawn grub killer: "worst case" user exposure and dose with long sleeved shirt and long pants, based on top of range data

Estimated top of range user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal	11705	234	936
Inhalational	0.63	0.63	0.63
TOTAL	11706	235	937
Bw-adjusted systemic dose (µg/kg)		**3.67**	**15**
Systemic dose as % of ARfD		37	150
Systemic dose as % of ADI		46	188

Note: Extrapolating from top of range data obtained by Merricks (1997b), total exposure would be reduced to approximately 615 µg if gloves were worn in addition to long pants and a long sleeved shirt.

Lawn grub killer: typical user exposure and dose with short sleeved shirt and short pants, based on mean data

Estimated mean user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal:	40	0.8	3.2

lower arms			
Dermal: lower legs	1488	30	120
Dermal: rest of skin	1253	43	172
Inhalational	0.23	0.23	0.23
TOTAL	2781	74	296
Bw-adjusted systemic dose (µg/kg)		**1.16**	**4.6**
Systemic dose as % of ARfD		12	46
Systemic dose as % of ADI		15	58

Lawn grub killer: "worst case" user exposure and dose with short sleeved shirt and short pants, based on top of range data

Estimated top of range user exposure, µg		**Estimated µg uptake over 30 min (2% dermal penetration)**	**Estimated µg uptake over 2 h (8% dermal penetration)**
Dermal: lower arms	7593	152	607
Dermal: lower legs	20303	406	1624
Dermal: rest of skin	11705	234	936
Inhalational	0.63	0.63	0.63
TOTAL	39602	793	3168
Bw-adjusted systemic dose (µg/kg)		**12**	**50**
Systemic dose as % of ARfD		120	500
Systemic dose as % of ADI		150	619

The second scenario is exposure from treated turf. Household occupants making contact with the treated lawn could dislodge carbaryl residues and hence incur dermal exposure to the chemical. No data are available on the extent to which carbaryl can be transferred from grass to the skin or clothing. However, US EPA SOPs for Residential Exposure Assessments (1997) provide a standard method for estimating potential post-application doses in the absence of field data. The EPA assumes that on the day of application, 20% of the applied active constituent is available from grass as dislodgeable residue. The Australian product is applied at 120 g carbaryl per 400 m^2 lawn, ie, 0.3 g/m^2 or 30 $\mu g/cm^2$. If 20% of the residue can be dislodged, 6 $\mu g/cm^2$ would be transferred from the grass onto the skin or clothing of a person contacting the treated area. Thus, a 100 cm^2 patch of treated grass could deliver a 600 µg quantity of carbaryl, which is equivalent to the ARfD of 10 µg/kg, assuming an adult human bodyweight of 60 kg.

However, this does not take into account the limited dermal absorption of carbaryl, which is itself dependent on the duration of exposure. From the dermal absorption studies, if the transferred carbaryl remained on the skin for 1 h, approximately 4% of the dose would be absorbed systemically. Under these conditions, an adult would have to transfer the dislodgeable carbaryl residue from a 100 ÷ 0.04 = 2500 cm^2 area to achieve an ARfD. Given that the surface area of an average adult male is 19 400 cm^2, it is apparent that an ARfD could indeed be attained by sitting or lying on the treated surface, provided the transferred carbaryl was not washed off the skin immediately.

The only practical means of protecting household occupants from this hazard is to ensure that they keep their bare skin off the treated lawn until the applied carbaryl has either soaked into (and adsorbed onto) the underlying soil, or degraded. A label restraint recommending avoidance of skin contact with treated turf is therefore recommended.

Conclusions

The reviewer perceives major advantages with the presentation of this 60 g/L carbaryl insecticide, in that the user does not have to mix a concentrate or clean up equipment after use, thereby reducing the opportunity for exposure. Furthermore, automatic admixture of concentrate into the water stream assures that accidental or deliberate use of an "over strength" spray mixture is unlikely. Notwithstanding, there is clear potential for the applicator to receive a systemic dose that exceeds both the ARfD and ADI when treating a 200 m^2 lawn, mainly via exposure on the hands and lower legs. Particularly under "worst case" conditions while wearing short pants and sleeves, the achievable systemic doses are considered to be toxicologically significant, being up to 5 or 6-fold higher than the ARfD and ADI, respectively. Given that larger lawns are not uncommon in domestic premises, some users could become even more heavily exposed than predicted.

Extrapolation from the USA studies, however, shows that **a combination of long pants, sleeves and gloves would reduce exposure by at least 20-fold, which is more than sufficient to obviate any reasonably anticipated hazard.** This could be achieved by revising the label Safety Directions to state "When using the product wear cotton overalls buttoned to the neck and wrist [or equivalent clothing] and rubber gloves" (279 283 290 292b 312).

OCS's examination of potential dermal exposure from contact with treated turf suggests that carbaryl doses exceeding the ARfD could occur, although a 50 cm^2 area of bare skin would have to be contaminated, and carbaryl residues would have to remain on the skin for 1 h. Given that this product is intended for use in domestic premises, **it is considered advisable to warn users by a label statement to avoid bare skin contact with treated grass.**

User exposure model for 0.96 g/l EM ready-to-use tomato sprays

Reference data: The most relevant data were obtained by Merricks (1997b) during application of a ready-to-use 0.1% carbaryl liquid to vegetables by trigger pump pack, with and without gloves.

Application equipment: Product is sold in 750 mL trigger pump bottle.

Adjustment for carbaryl concentration in product: None required. Note that both products registered in Australia also contain 2.4 g/L sulfur and 1.4 g/L copper oxychloride.

Personal protective equipment: No safety directions have been set specifically for this type of combination ready-to-use EM product. The available product label directs users to wear rubber gloves when using the prepared spray. Systemic dose estimates have therefore been prepared from exposure data derived from persons wearing gloves.

1 g/L tomato spray: typical user exposure and dose with gloves, long sleeved shirt and long pants, based on mean data

Geometric mean user exposure, μg		Estimated μg uptake over 30 min (2% dermal penetration)	Estimated μg uptake over 2 h (8% dermal penetration)
Dermal	5.2	0.10	0.42
Inhalational	0.15	0.15	0.15
TOTAL	5.4	0.25	0.57
Bw-adjusted systemic dose (μg/kg)		**0.004**	**0.009**
Systemic dose as % of ARfD		0.04	0.09
Systemic dose as % of ADI		0.05	0.11

Note: Geometric mean data obtained by Merricks (1997b), total exposure would be increased to approximately 96 μg if gloves were **not** worn with long pants/long sleeved shirt.

1 g/L tomato spray: "worst case" user exposure and dose with gloves, long sleeved shirt and long pants, based on top of range data

Top of range user exposure, μg		Estimated μg uptake over 30 min (2% dermal penetration)	Estimated μg uptake over 2 h (8% dermal penetration)
Dermal	74	1.48	5.9
Inhalational	0.88	0.88	0.88
TOTAL	75	2.4	6.8
Bw-adjusted systemic dose (μg/kg)		**0.04**	**0.11**
Systemic dose as % of ARfD		0.4	1.1
Systemic dose as % of ADI		0.5	1.4

Note: Extrapolating from top of range data obtained by Merricks (1997b), total exposure would be increased to approximately 729 μg if gloves were **not** worn with long pants/long sleeved shirt. This would deliver a 0.23 or 0.91 μg/kg dose to an adult after 30 min or 2 h, respectively.

1 g/L tomato spray: typical user exposure and dose with gloves, short sleeved shirt and short pants, based on mean data

Geometric mean user exposure, μg		Estimated μg uptake over 30 min (2% dermal penetration)	Estimated μg uptake over 2 h (8% dermal penetration)
Dermal: lower arms	2.3	0.05	0.20
Dermal: lower legs	14.1	0.28	1.12
Dermal: rest of skin	5.2	0.10	0.42
Inhalational	0.15	0.15	0.15
TOTAL	21.8	0.58	1.89
Bw-adjusted systemic dose (μg/kg)		**0.009**	**0.029**

Systemic dose as % of ARfD	0.09	0.29
Systemic dose as % of ADI	0.12	0.37

1 g/L tomato spray: "worst case" user exposure and dose with gloves, short sleeved shirt and short pants, based on top of range data

Top of range user exposure, μg		**Estimated μg uptake over 30 min (2% dermal penetration)**	**Estimated μg uptake over 2 h (8% dermal penetration)**
Dermal: lower arms	13	0.26	1.04
Dermal: lower legs	96	1.92	7.7
Dermal: rest of skin	74	1.48	5.9
Inhalational	0.88	0.88	0.88
TOTAL	184	4.5	16
Bw-adjusted systemic dose (μg/kg)		**0.07**	**0.24**
Systemic dose as % of ARfD		0.70	2.4
Systemic dose as % of ADI		0.88	3.0

Conclusion

Under the existing conditions of use, **dermal exposure sufficient to deliver a toxicologically significant dose would be improbable**. A new entry should therefore be established in the FAISD Handbook for this product class, including statements 279 283 290 312 ("When using the product wear rubber gloves").

[NOTE: Safety directions for this product category will be reviewed following the forthcoming APVMA review of carbaryl residues in food.]

Householder exposure from application of carbaryl to pathways

In addition to its use for plant protection in the home garden, carbaryl is also used for control of Portuguese millipedes and some other crawling arthropods on garden beds, compost heaps and around the exterior of houses. The product registered for this purpose is an 800 g/kg WP available only to the commercial market sector; as such, it would be applied by pest control operators, and so householder exposure should be limited to post-application contact with treated surfaces.

For millipede control, the product is diluted to 10 g/L water (ie 8 g/L carbaryl) and sprayed onto paths around buildings and walls to the height of 1 m, forming a protective chemical barrier. A 5L volume of spray mixture (containing 40 g carbaryl) is sufficient to cover an area of 30 m^2, delivering a surface concentration of 1.25 g carbaryl/m^2 or 125 μg/cm^2. The US EPA (1997) assumes that 50% of pesticide residue on a hard surface is dislodgeable, and so 62.5 μg/cm^2 would be transferred from the path onto the skin or shoes of a person contacting the treated area. Thus, a 9.6 cm^2 area of treated path could deliver a 600 μg quantity of carbaryl, which is equivalent to the ARfD of 10 μg/kg, assuming an adult human bodyweight of 60 kg.

Taking into account the limited dermal absorption of carbaryl, if the transferred carbaryl remained on the skin for 1 h, approximately 4% of the dose would be absorbed systemically. Under these conditions, an adult would have to transfer the dislodgeable carbaryl residue from a 9.6 ÷ 0.04 = 240 cm^2 area to achieve an ARfD. Given that the surface area on the sole of an adult's foot is approximately 200 cm^2, it is apparent that an ARfD could indeed be attained by standing or walking on the treated surface, provided the transferred carbaryl was not washed off the skin immediately.

The only practical way to protect household residents from exposure to carbaryl from this source, is to ensure they do not make contact with treated paths until the chemical has degraded sufficiently. No information is available on the persistence of carbaryl on hard outdoor surfaces, and so a specific time interval cannot be nominated.

Conclusion:

Carbaryl products intended for application around the exterior of domestic premises should bear a label statement advising that bare skin contact should not be made with treated areas following application.

Summary table: estimated combined dermal and inhalational exposure to carbaryl of persons applying home veterinary products, shown as proportion of toxicological benchmarks

Systemic exposure estimates that exceed the ADI or ARfD are shown highlighted.

Exposure scenario	Duration of dermal exposure (h)	% of ARfD (10 µg/kg/d)	% of NOEL for ChE inhibition (1000 µg/kg/d)	% of ADI (8 µg/kg/d)	% of LOEL for tumours (16000 µg/kg/d)
50 g/kg pet dusts – treatment of 1 medium sized dog					
Typical exposure, long pants & sleeves	0.5	16	0.16	21	0.01
	2.0	40	0.4	50	0.025
Top of range exposure, long pants & sleeves	0.5	90	0.9	117	0.058
	2.0	210	2.1	265	0.13
Typical exposure, short pants & sleeves	0.5	25	0.25	32	0.016
	2.0	80	0.8	95	0.047
Top of range exposure, short pants & sleeves	0.5	200	2	253	0.13
	2.0	650	6.5	812	0.41
50 g/kg pet dusts – treatment of aviary					
Typical exposure, long pants & sleeves	0.5	33	0.33	42	0.021
	2.0	82	0.82	103	0.051

Top of range exposure, long pants & sleeves	0.5 2.0	190 420	1.9 4.2	233 525	0.12 0.26
Typical exposure, short pants & sleeves	0.5 2.0	51 150	0.51 1.5	64 188	0.032 0.094
Top of range exposure, short pants & sleeves	0.5 2.0	400 1300	4 13	500 1625	0.25 0.81
10 g/L ear drops – accidental spill during treatment					
Typical exposure, confined to hand	0.5 2.0	31 125	0.31 1.25	39 157	0.020 0.078
120 g/kg pet collar – unwrapping and fitting					
Typical exposure	0.25	67	0.67	84	0.042
10 g/L pet shampoo - application					
Typical exposure	0.25	32	0.32	40	0.020

Exposure scenario	Duration of dermal exposure (h)	% of ARfD (10 µg/kg/d)	% of NOEL for ChE inhibition (1000 µg/kg/d)	% of ADI (8 µg/kg/d)	% of LOEL for tumours (16000 µg/kg/d)
50 g/kg dusts – treatment of garden and vegetables					
Typical exposure, long pants & sleeves	0.5 2.0	2.8 8.2	0.028 0.082	3.5 11	0.002 0.005
Top of range exposure, long pants & sleeves	0.5 2.0	30 93	0.30 0.93	38 117	0.019 0.059
Typical exposure, short pants & sleeves	0.5 2.0	3.2 9.9	0.032 0.099	4.0 13	0.002 0.006
Top of range exposure, short pants & sleeves	0.5 2.0	35 113	0.35 1.13	44 142	0.022 0.071
20 g/kg dusts – treatment of garden and vegetables					
Typical exposure, long pants & sleeves	0.5 2.0	1.1 3.3	0.011 0.033	1.4 4.1	0.0007 0.002
Top of range exposure, long pants & sleeves	0.5 2.0	8.3 37	0.083 0.37	17 47	0.008 0.024

Typical exposure, short pants & sleeves	0.5 2.0	1.3 4.0	0.013 0.04	1.5 5	0.0008 0.003
Top of range exposure, short pants & sleeves	0.5 2.0	14 45	0.14 0.45	18 57	0.009 0.028
Wettable powders – treatment of fruit trees and ornamentals					
Typical exposure, long pants & sleeves	0.5 2.0	1.7 6.6	0.017 0.066	2.2 8.3	0.001 0.004
Top of range exposure, long pants & sleeves	0.5 2.0	9.8 39	0.10 0.39	12 49	0.006 0.025
Typical exposure, short pants & sleeves	0.5 2.0	11 44	0.11 0.44	14 55	0.007 0.027
Top of range exposure, short pants & sleeves	0.5 2.0	17 67	0.17 0.67	21 84	0.011 0.042
Wettable powders – treatment of vegetables					
Typical exposure, long pants & sleeves	0.5 2.0	0.8 3	0.008 0.03	1 3.5	0.0005 0.002
Top of range exposure, long pants & sleeves	0.5 2.0	6.7 27	0.067 0.27	8 34	0.004 0.017
Typical exposure, short pants & sleeves	0.5 2.0	1.4 5.5	0.014 0.055	1.8 7	0.0009 0.004
Top of range exposure, short pants & sleeves	0.5 2.0	19 75	0.19 0.75	24 94	0.012 0.047
60 g/L hose on insecticide – treatment of turf					
Typical exposure, long pants & sleeves	0.5 2.0	6.7 27	0.067 0.27	9 34	0.004 0.017
Top of range exposure, long pants & sleeves	0.5 2.0	37 150	0.37 1.5	46 188	0.023 0.094
Typical exposure, short pants & sleeves	0.5 2.0	12 46	0.12 0.46	15 58	0.007 0.029
Top of range exposure, short pants & sleeves	0.5 2.0	120 500	1.2 5	150 619	0.075 0.31
0.96 g/L ready-to-use spray – application to tomatoes					
Typical exposure, long pants & sleeves	0.5 2.0	0.04 0.09	0.0004 0.0009	0.05 0.11	0.00003 0.00005

Top of range exposure, long pants & sleeves	0.5 2.0	0.4 1.1	0.004 0.011	0.50 1.4	0.0003 0.0007
Typical exposure, short pants & sleeves	0.5 2.0	0.09 0.29	0.0009 0.0029	0.12 0.37	0.00006 0.0002
Top of range exposure, short pants & sleeves	0.5 2.0	0.7 2.4	0.007 0.024	0.90 3	0.0005 0.0015

1.5 HAZARD ASSESSMENT

1.5.1 Dietary intake from home grown commodities

A number of carbaryl-based products are registered for HG use on fruit and vegetables. The table below summarises their use patterns. The working strength of products applied by spray is 1 g carbaryl/L. Most of the HG product labels recommend a 3-day interval between treatment and consumption of edible commodities.

Use patterns of carbaryl HG products intended for application to food plants

Situation	Product	Application instructions
External areas, vegetables, flowers, ornamentals	DU containing 50 g/kg carbaryl in shaker dispenser.	Vegetables: Dust lightly, covering all plant surfaces at 7-10 d intervals.
Flowers, ornamentals, vegetables including cabbages, cauliflowers, turnips, Brussels sprouts, broccoli, tomatoes	DU products containing 19 – 20 g/kg carbaryl. Usually in shaker dispenser, some with additional active constituents.	Dust lightly, thoroughly covering all plant surfaces at 7-10 d intervals (some products) ranging up to 2-3 wk between applications. Reapply dust when removed by rain or watering. A 3-d WHP specified for vegetables.
Apples, pears, peaches, plums, nectarines, vegetables, ornamentals	WP containing 800 g/kg carbaryl in 200 g, 500 g & 1 kg pack sizes. Dilution rate: 1 g carbaryl /L water.	Vegetables: spray when insects first appear and then about every 7-10 d as necessary. Fruit: Apply about every 3 wk from mid September. A 3-d WHP applies.
Tomatoes	WP containing 80 g/kg carbaryl with 200 g/kg sulfur & 120 g/kg copper. Packed in 60 g measure packs, to be diluted with 5 L water.	Spray at 7 d intervals up until and after transplanting. A 3-d WHP is specified.
Tomatoes	Ready to use LD containing 0.96 g/L carbaryl with 2.4 g/L sulfur and 1.44 g/L copper.	Spray plants thoroughly every 7 d.
Roses, flowers, ornamentals, fruit trees, vegetables	WP containing 100 g/kg carbaryl, 300 g/kg sulfur & 135 g/kg mancozeb. Used at 1 g carbaryl/L	Apply when disease or pest first appears and then every 14 d as necessary. 14-d WHP for beetroot, leafy vegetables, grapes, tomatoes, pome & stone fruit. 7-d WHP for other edible crops.
Ornamentals, apples,	LD / AC products containing	Vegetables and ornamentals: apply at first sign of pest

pears, peaches, plums, nectarines, vegetables and lawns	400-500 g/L carbaryl. Used at 1 g carbaryl/L	activity then every 7-10 d as necessary. Stone fruit: spray 3 wk after petal fall. Repeat every 3-4 wk. A 3-d WHP applies to edible crops.
Ornamentals, vegetables, fruit trees, apples, pears, citrus, grapes, turf, areas outside houses & sheds	AC, SC, EC or OL products containing 100 g/L carbaryl. Diluted to 10 mL/L (1 g carbaryl/L) for application to fruit and vegetables.	Fruit trees: Spray 3 wk after petal fall. Repeat every 3 wk. Grapes: Apply 2 wk after bud burst, repeat as necessary. Vegetables: Spray when insects first appear and then every 7-10 d to 3 wk as necessary. A 3-d WHP applies.

The OCS has considered uses of carbaryl on food producing plants in the home garden and finds that irrespective of the application method, there are additional factors that would tend to increase both the concentration of carbaryl residues on home-grown produce, and the amount of treated home-grown produce consumed, relative to commercially-grown commodities. Compared with professional users, there is a higher probability that domestic users may deliberately or mistakenly apply excessive amounts of carbaryl, or may ignore the recommended 3-day withholding period for treated commodities. Secondly, home-grown fruits and vegetables do not have to pass through the commercial "chain of supply" to reach the consumer, thereby reducing the time interval over which carbaryl could degrade between treatment and consumption of commodities. Thirdly, there will be marked seasonal peaks in the consumption of treated garden produce, which will substitute for alternative commodities from commercial sources.

Hence, given that the high (97.5^{th} percentile) consumer exposure model would apply in the home garden setting and that carbaryl residues in home-grown produce would probably equal or exceed the levels measured under supervised trial conditions, the NESTI values calculated by the APVMA are highly relevant to consumers of home-grown commodities treated with carbaryl. It is considered that there is equal or greater potential for dietary intake of carbaryl residues in home-grown commodities to exceed the ARfD, as compared with residues in commercially grown produce.

Furthermore, while NESTIs are predicated on exposure in one meal or a single day, people will consume home-grown commodities repeatedly for several weeks or months. This scenario resembles the pattern of sustained dietary exposure, which underlies the ADI concept. As the ADI for carbaryl is 0.008 mg/kg bw/d, the findings of the APVMA Residues Evaluation Report indicate the potential for significant, sustained and unacceptable erosion of the 2000-fold safety factor applied to the LOEL for vascular tumour formation in mice (16 mg/kg bw/d).

Although HG use of carbaryl on raspberries, turnip, sugarbeet, beetroot, potato, macadamias and pecans may be supportable from a toxicological standpoint, restriction of HG food uses to these particular commodities may prove ineffective because of non-compliance. It is highly probable that some users would continue to apply carbaryl to the commodities that have been shown to retain sufficient carbaryl residues to cause dietary intake exceeding the ARfD and ADI by a wide margin. Consequently, the OCS recommends that the HG use of carbaryl on food plants should be discontinued, as there are insufficient grounds to provide assurance that such use will not be likely to have an effect that is harmful to human beings.

1.5.2 Intake from Drinking Water

Current registered uses of carbaryl include cotton and rice. Where a pesticide is registered for use in water or water catchment areas, the Joint Committee of the Agricultural and Resource Management Council of Australia and New Zealand and the NHMRC set Guideline and Health Values for the chemical in drinking water. A Guideline Value is generally based on the analytical limit of determination, and is set at a level consistent with good water management practice and that would not result in any significant risk to the consumer over a lifetime of consumption. Exceeding the Guideline Value indicates undesirable contamination of drinking water and should trigger action to identify the source of contamination and prevent further contamination. However, a breach of the Guideline Value does not necessarily indicate a hazard to public health. The current Guideline Value for carbaryl is 0.005 mg/L.

Health Values are intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or mis-use of a pesticide. The values are derived so as to limit intake *from water alone* to about 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg at a daily water consumption of 2 L over a lifetime. At present, the Health Value for carbaryl is 0.03 mg/L (*Australian Drinking Water Guidelines - Summary*, NHMRC, Canberra, Australia,1996; ISBN 0 642 24462 6 or http://www.nhmrc.gov.au/publications/pdf/eh20.pdf).

Given that the ADI for carbaryl is 0.008 mg/kg bw/d, the Health Value may be calculated as:

$$\frac{0.008 \text{ mg/kg bw/d} \times 70 \text{ kg} \times 0.1}{2 \text{ L/d}}$$

$$= 0.028 \text{ mg/L}$$

Hence, the current Health Value for carbaryl of 0.03 mg/L is supported, and no revision is proposed.

1.5.3 Home veterinary and home garden uses of carbaryl in non-food producing situations

The OCS re-affirms its previous recommendation for withdrawal from registration of carbaryl-based HV dusts intended for treatment of animals and birds, all carbaryl products intended for indoor use, and HG products containing more than 160 g/kg (or g/L) carbaryl.

The OCS has no objections on toxicological grounds to the continuing registration of HG products containing up to 160 g/kg of carbaryl for use in non-food producing situations including ornamental plants. As discussed in the May 2003 OCS evaluation, exposure studies have demonstrated that carbaryl plant dusts, wettable powders and liquids cause negligible exposure to users by inhalation when applied as directed. A combination of long pants, long sleeves and gloves is sufficient to limit dermal exposure and uptake to levels well below the ADI and ARfD for carbaryl, and therefore these products may be used safely provided appropriate warning statements and Safety Directions appear on the product labels (see below).

1.5.4 Additional data

During preparation of this review, examination of the US EPA Revised HED Risk Assessment on carbaryl (2003a) has revealed the existence of several studies on carbaryl that have not been submitted to the Australian regulatory authorities. These include 3 studies on the dermal toxicity of carbaryl in rats, studies on residue transfer from treated turf and pet collars, and a biomonitoring study of absorbed dose levels of carbaryl in persons whose lawns and gardens were treated with the chemical. Given that the data appear to be directly relevant to the health and safety of persons using carbaryl products or residing in treated premises, these studies are required for review by the OCS, and should be requested from the registrant.

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2 RESIDUES EVALUATION REPORT

2.1 INTRODUCTION

The review of carbaryl was initiated in 1995, as it was considered that the APVMA at that time did not hold sufficient residue data to support the use of carbaryl in cereals, either by field application or for use on stored grain. The scope of the review for carbaryl initially included the reconsideration of residue data and MRLs related to cereal grains and animals that may be fed on treated cereal products. The scope of the review was later extended to include home garden and home veterinary products (NRA Gazette No. 7, 6 July 1999).

In 2001 the JMPR set an ARfD for carbaryl, and the Office of Chemical Safety (OCS) recommended an ARfD in December 2002. As a consequence of the establishment of an ARfD by the OCS, the scope of the carbaryl special review was again broadened, so that the APVMA could assess the acute dietary risk of carbaryl (APVMA Gazette No. 6, 3 June 2003).

2.2 DISCUSSION

Residue trial data was submitted in support of the use of carbaryl in cereal grains, with additional data presented on residues in animal feed commodities and crops for human consumption. The APVMA or the Pesticides and Agricultural Chemicals Committee (PACC) had not previously evaluated most of the data presented.

Previous evaluations of the plant and animal metabolism data for carbaryl have resulted in the establishment of parent compound as the residue definition, both in Australia and internationally *via* Codex Alimentarius Commission (CODEX). The plant and animal metabolism of carbaryl, and analytical methodology, were most recently evaluated by the JMPR in 2002.

2.3 MAXIMUM RESIDUE LIMITS (MRLs)

Current Australian MRLs for carbaryl are tabulated in Table 1. Most of the Australian MRLs for carbaryl were adopted from CODEX by the Pesticide and Agricultural Chemicals Standing Committee (PACC).

Table 1 *MRL Standard* entries

Code		Food	MRL (mg/kg)
FS	0240	Apricot	10
VS	0621	Asparagus	10
FI	0326	Avocado	10
FI	0327	Banana [in the pulp]	5
FB	0264	Blackberries	10
FB	0020	Blueberries	7
FT	0289	Carambola	5
GC	0080	Cereal grains	T5
FS	0013	Cherries	5
FC	0001	Citrus fruits	7
SO	0691	Cotton seed	1
FI	0332	Custard apple	5
FB	0266	Dewberries (including Boysenberry and Loganberry)	10

MO	0105	Edible offal (mammalian)	T0.2
PE	0112	Eggs	T0.2
FI	0371	Elephant apple	5
FI	0335	Feijoa	5
VC	0045	Fruiting vegetables, Cucurbits	3
FI	0351	Granadilla	5
FB	0269	Grapes	5
FT	0298	Grumichama [Brazilian cherry]	5
FT	0336	Guava	5
FT	0300	Jaboticaba	5
FI	0338	Jackfruit	5
		Jambu	5
FI	0341	Kiwifruit	10
VL	0053	Leafy vegetables	10
FI	0343	Litchi	5
FI	0342	Longan	5
FI	0345	Mango	5
MM	0095	Meat [mammalian]	T0.2
ML	0106	Milks	T*0.05
FS	0245	Nectarine	10
VO	0442	Okra	10
FT	0305	Olives	10
DM	0305	Olives, processed	1
FI	0350	Papaya [pawpaw]	5
FI	0351	Passion fruit	5
FS	0247	Peach	10
FS	0014	Plums (including Prunes)	5
FP	0009	Pome fruits	5
VR	0589	Potato	0.2
PO	0111	Poultry, Edible offal of	T5
PM	0110	Poultry meat	T0.5
FI	0358	Rambutan	5
FB	0272	Raspberries	10
FI	0359	Sapodilla	5
FI	0360	Sapote, Black	5
FI	0361	Sapote, Green	5
FI	0362	Sapote, Mammey	5
FI	0363	Sapote, White [casimiroa]	5
FB	0275	Strawberry	7
GS	0659	Sugar cane	T*0.05
SO	0702	Sunflower seed	1
VO	0447	Sweet corn (corn-on-the-cob)	1
TN	0085	Tree nuts	1
TN	0085	Tree nuts [whole in shell]	10
		Vegetables [except asparagus; fruiting vegetables, cucurbits; leafy vegetables; okra; potato; sweet corn (corn-on-the-cob)]	5
CM	0654	Wheat bran, unprocessed	T20

Table 4 *MRL Standard* entries

Code		Animal feed commodity	MRL (mg/kg)
AF	0080	Forage of cereal grains	T100
AS	0081	Straw and fodder (dry) of cereal grains	T100

There are no current Table 5 *MRL Standard* entries for carbaryl.

2.4 DIRECT VETERINARY APPLICATION OF CARBARYL TO POULTRY

One product, KEYDUST Dusting Powder (46851), is registered for control of ectoparasites on poultry.

The maximum application rate is 30 kg product/1500 m^2 (average broiler shed), which is equivalent to 1200 g carbaryl/1500 m^2. Assuming that the average broiler shed contains 30 000 birds, this equates to an average exposure rate of 40 mg carbaryl/bird. A bird weighing 2 kg is estimated to consume 150 g dry matter per day. Thus, an exposure rate of 40 mg carbaryl/bird/day is estimated to be equivalent to a feeding level of 265 ppm (dry weight basis). Forty mg carbaryl/bird is also estimated to be equivalent to a direct application rate of 1 g of dust (product)/bird.

2.4.1 Residues data considered

The residues aspects of carbaryl have been reviewed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) on numerous occasions. The relevant residues data for carbaryl in poultry are reproduced below.

JMPR (1973): Following administration of l-naphthyl-^{14}C-carbaryl to hens, total ^{14}C-residues reached a maximum and dissipated at a much faster rate in egg white than in egg yolk. In a single dose of 10 mg/kg (Paulson and Foil, 1969)[1], maximum concentration of ^{14}C-residues in egg white was 0.12 ppm at one day and dropped to trace amounts on the second day after treatment. The yolk residues reached a maximum at the fifth day (0.36 ppm) and had dissipated by the ninth (0.03 ppm). Under continuous feeding conditions, the total residue in the yolk or white at each sampling time was dosage related (Andrawes *et al*., 1972[2]). Concentration of ^{14}C-carbaryl equivalents (ppm) reached a maximum (0.10 ppm from 70 ppm in feed; 0.025 ppm from 21 ppm in feed) in the white after two to six days and in the yolk (1.0 ppm from 70 ppm in feed; 0.30 ppm from 21 ppm in feed) after six to nine days of dosing and remained level until the end of the treatment period. At plateau levels, the level of ^{14}C-carbaryl equivalents in the white was one-tenth that in the yolk; however, the total equivalents were in a ratio of 5:1 between yolk and white. The ratio of the concentration of carbaryl in whole eggs (white and yolk) to that in the diet was 0.006 at equilibration. After discontinuation of dosing, residues in the whites had a half-life of less than one day; for yolk residues the half-life was approximately two to three days.

[1] Paulson, G.D. and Feil, V.J. (1969) *Poultry Sci*. 48: 1593

[2] Andrawes, N.R., Chancey, E.L., Crabtree, R.J. Herrett, R.A. and Weiden, M.H.J. (1972). 'Fate of Napthyl-1-^{14}C-Carbaryl in Laying Chickens'. *Journal of Agricultural Food Chemistry*, 20, 608–617.

The distribution of carbaryl residues was determined in hen tissues after continuous treatment with either 7, 21 or 70 ppm of l-naphthyl-^{14}C-carbaryl in the diet (Andrawes *et al*., 1972)[3]. Tissue residues were directly proportional to the concentration of carbaryl in the diet. The highest residues were found in the blood and tissues of high blood content (liver, kidney, lung and spleen); body fat, brain and muscles contained the lowest residues. For example, the distribution of ^{14}C-carbaryl equivalents one day after treatment for 14 days with 70 ppm in the diet was as follows (in ppm): liver 0.41, kidney 0.485, thigh 0.03, leg 0.032, breast 0.031, skin 0.043, fat 0.026, gizzard 0.04, heart 0.049, and brain 0.017. The half-life of total body residues was calculated to be five days.

JMPR (1976): In continuous feeding studies with radio-labelled carbaryl (Andrawes *et al*., 1972), residues reached maximum levels within one day in the excrement, two days in egg white and six to eight days in egg yolk. The residues in the whole egg (yolk plus white) were directly proportional to the amount of carbaryl fed. An intake of 7 mg/kg of carbaryl in the feed resulted in a residue of 0.04 mg/kg carbaryl equivalents in the whole egg. Radio-labelled residues in the excrement 15 hours after the initial treatment reached 80–100 per cent of the dose. Within one day after the discontinuation of dosing, the highest tissue residues were found in the excretory organs while very low residues were found in the fat indicating that carbaryl residues are not stored in body tissues. This work shows that carbaryl is metabolised in laying hens by pathways similar to those in mammals.

JMPR (1984): Following the request of the Codex Committee on Pesticide Residues (CCPR), information concerning the use of carbaryl on or near poultry was received by FAO from several countries.

- In Canada, carbaryl is the major pesticide used for direct application to poultry for control of mites. It is also approved for direct application to poultry against lice and as a supplement to premise treatment for chicken mites, fleas and fowl ticks. Carbaryl spray and dust is applied directly to poultry at the rate of 22.5 g/100 birds. Carbaryl dust (5 per cent) is applied to poultry dusting boxes at the rate of 120 g ai/100 birds. The treatment is not to be made within seven days of slaughter (Canada, 1984[4]).
- In the Netherlands, carbaryl is approved for the treatment of pens, sheds and other structures for the control of chicken mites, lice, mealworms and fleas. A suspension containing 5 g/L is used at the rate of 1 L/35 sq m. Carbaryl dust was authorised for direct application to poultry but was withdrawn in 1980 because of unpredictable residues in meat and eggs (Netherlands, 1984[5]).
- In Portugal, carbaryl is approved for direct application to poultry as a 5 per cent dust, with or without pyrethrum. An interval of two weeks between treatments is recommended, with an interval of seven days between the last application and slaughter (Portugal, 1984[6]).
- In the United States, carbaryl suspension concentrates, wettable powders and dusts may be applied directly to poultry for the control of northern fowl mite, chicken mite, lice and fleas. The dust is applied at the rate of 500 g/100 birds and 0.5 per cent sprays at the rate

[3] Andrawes, N.R., Chancey, E.L., Crabtree, R.J. Herrett, R.A. and Weiden, M.H.J. (1972). 'Fate of Napthyl-1-^{14}C-Carbaryl in Laying Chickens'. *Journal of Agricultural Food Chemistry*, 20, 608–617.

[4] Canada (1984). Information on use patterns of carbaryl in poultry submitted 1984 by Canada to FAO.

[5] Netherlands (1984). Information on use patterns of carbaryl in poultry 1984 submitted by the Netherlands to FAO.

[6] Portugal (1984) Information on use patterns of carbaryl in poultry submitted 1984 by Portugal to FAO.

of 4 L/100 birds. Carbaryl dust (5 per cent) is used in dust baths at the rate of 1 kg per box for each 50 birds. There is a seven-day interval between the last application and day of slaughter. The relative proportion of dust and spray is not known (United States, 1984[7]).

The following summary of results from three residue trials on laying hens and poultry poults indicates the level and distribution of carbaryl residues in poultry tissues (Union Carbide, 1984[8]).

In the first trial, laying hens were dusted with 4 g of 5 per cent dust per bird (recommended rate) three times at four day intervals (once in 28 days is recommended) and slaughtered at one and seven days after the last treatment. Samples of skin, breast muscle, leg muscle and liver were taken from each of six hens and separately analysed following each slaughter. The colorimetric method of Johnson *et al*. (1963)[9] was used to determine carbaryl and 1-naphthol separately at a method sensitivity of 0.1 to 0.2 mg/kg. Residues of 1-naphthol were less than 10 per cent of carbaryl residues in every case. Results below (see Table) are averages of duplicate analyses on each bird.

Averages of duplicate results of carbaryl residues in laying hens

Tissue	Residues at 1 day post-treatment (mg/kg)		Residues at 7 days post-treatment (mg/kg)	
	Maximum	Average	Maximum	Average
Chicken skin	35.0	19.3	3.1	2.2
Breast muscle	1.1	0.4	0.1	<0.2
Leg muscle	2.0	0.9	0.1	0.1
Liver	0.2	<0.2	<0.2	<0.2

In the second trial, turkey poults at two weeks of age were dusted with 5 per cent carbaryl three times at 14-day intervals, using a squeeze bottle applying 1, 2 and 3 g/bird, successively. Sprays of 0.5 per cent were applied at the same time using 1 and 1.5 mL/bird. Sampling and analyses were performed as per the first trial. Results below (see Table) are averages of duplicate analyses on each bird.

Averages of duplicate results of carbaryl residues in turkey poults

Tissue	**Carbaryl residues (mg/kg)**	
	1 day post-treatment	**7 days post-treatment**
Dusted		
Skin	0.99	1.06
Breast	0.64	2.07
Liver	1.89	1.64
Sprayed		
Skin	1.59	0.96
Breast	0.09	1.18

[7] United States (1984). Information on use patterns of carbaryl in poultry 1984 submitted by the United States to FAO.
[8] Union Carbide (1984). Information on residues of carbaryl in poultry 1984 submitted by Union Carbide Agricultural Products Company to FAO.
[9] Johnson, D.P., Critchfield, F.E. & Arthur, B.W. (1963). Determination of Sevin insecticide and its metabolites in poultry tissues and eggs. *J. Agric. Food Chem*., 11:77-80.

In the third trial, mature hens were treated using dust/bath boxes employing 5 per cent carbaryl dust. Sampling and analyses were performed as per the first trial. Results below **(**see Table) are averages of duplicate analyses on each bird.

Averages of duplicate results of carbaryl residues in mature hens

Tissue	Average carbaryl residues (mg/kg)		
	7 days post-treatment	14 days post-treatment	28 days post-treatment
Breast	<0.2	<0.2	<0.2
Skin	0.96	0.37	0.08
Liver	<0.2	<0.2	<0.2

JMPR (2002): The metabolism of carbaryl in hens was studied after oral administration of 1-naphthyl-^{14}C carbaryl to laying hens treated twice a day for seven consecutive days at 8.8 ppm and 10.5 ppm of carbaryl in the diet. On average, 97.7 per cent of the radioactivity was recovered in the excreta. Tissues contained only 0.17 per cent of the administered dose, mostly concentrated in kidney (0.268 mg equiv./kg) and liver (0.187 mg equiv./kg). Egg yolk contained up to 0.176 mg equiv./kg, with 1-naphthol sulphate being the major metabolite (0.078 mg equiv./kg). Desmethylcarbaryl was the major metabolite in liver (0.017 mg equiv./kg), and 1-naphthol was the major metabolite in abdominal fat (39.1 mg equiv./kg). The highest concentration of free carbaryl was found in fat (26.9 per cent of total radioactive residues (TRR), 0.004 mg equiv./kg).

For poultry, the maximum and supervised trial median residue (STMR) estimated dietary burden were 34.4 and 6.4 mg/kg feed, respectively. Metabolism studies on hens conducted at 8.8 and 10.5 mg/kg feed (seven consecutive days orally dosed) showed detectable residue of carbaryl in egg yolks, liver and abdominal fat (0.001 to 0.004 mg/kg ^{14}C-carbaryl equivalents). The JMPR agreed that this study is not adequate to estimate maximum residue levels of carbaryl in poultry.

2.4.2 Residues discussion

Carbaryl residues in tissues from treated birds

It is noted that the registered (overseas) use patterns of carbaryl dust on poultry (as reported by JMPR, 1984) appear to be significantly higher than the corresponding Australian use patterns: the overseas dose rates range from 150 to 250 mg carbaryl/bird, and incorporate a seven-day withholding period (WHP). In contrast, the estimated Australian exposure rate is ~40 mg carbaryl/bird with nil meat and egg WHPs.

When laying hens were dusted with three applications of 200 mg carbaryl/bird at four-day intervals, maximum residues in edible chicken tissues at one day after the last treatment were 35 mg/kg for skin, 2.0 mg/kg for muscle and 0.2 mg/kg for liver. Assuming a linear correlation between dose rate and residue concentration, correction to the 1× dose rate of 40 mg carbaryl/bird gives residues of 7 mg/kg for skin, 0.4 mg/kg for muscle and 0.04 mg/kg for liver.

In a second study where turkey poults were dusted three times at 14-day intervals with 50, 100 and 150 mg carbaryl/bird (successive applications), average carbaryl residues at one day after the last treatment were 0.99 mg/kg for skin, 0.64 mg/kg for breast muscle, and 1.89 mg/kg for liver. Correction of these results to the 1× rate of 40 mg carbaryl/bird gives residues of 0.26 mg/kg for skin, 0.17 mg/kg for muscle and 0.50 mg/kg for liver. It is also noted that the levels of carbaryl

residues in skin and breast muscle were higher at seven days post-treatment than at one day post-treatment.

Based on the available residues data, it is clear that direct (veterinary) application of carbaryl-based dust products on poultry is likely to result in residue levels that exceed the poultry MRLs, 0.2 mg/kg for poultry offal and *0.02 mg/kg for poultry meat.

Carbaryl residues in eggs from treated birds

The amount of residues data for eggs from hens that were treated directly with carbaryl (via dusting) is very limited. In one study conducted by Schenck *et al*. (2003)[10], hens were dusted once with 10 per cent carbaryl powder (application rate not provided). Eggs from treated birds contained 0.076 mg/kg at one day post-treatment, and residues declined to 0.054 mg/kg at two days post-treatment, and 0.042 mg/kg at six days post-treatment. These results indicate that the direct application of carbaryl-based dust products on laying hens is likely to result in residue levels that exceed the egg MRL of *0.02[11] mg/kg.

2.4.3 Conclusion

Based on the available residues data, it is concluded that the use of carbaryl on poultry, as per the directions on the product label for KEYDUST Dusting Powder (P46851), is likely to result in residues in edible poultry commodities that exceed the MRLs (ie 0.2 mg/kg for poultry offal, *0.02 mg/kg for poultry meat, and *0.02 mg/kg for eggs).

The available data are not considered adequate to enable revision of the MRL recommendations (to cover the direct dust application to poultry), since none of the trials addressed the maximum Australian use rate. Furthermore, studies show that levels of carbaryl residues in skin and breast muscle were higher at seven days post-treatment than at one day post-treatment. The Australian use pattern is associated with nil meat and egg WHPs, which means that the MRLs need to cover residue levels at all times post-treatment.

2.5 RESIDUES IN FRUITS AND VEGETABLES

Residues trial data were provided for a wide variety of fruit and vegetable crops. The data was presented as either full studies, brief trial reports or summary tables of results. Results from all of the trial information provided to the APVMA were used to determine the levels of residues expected to occur in commodities treated according to Australian good agricultural practice (GAP) and, where required, results were scaled down to reflect the maximum approved Australian application rates. The trials available for review are included in the Appendices to this report and results are summarised in the table below.

[10] Schenck, F.J., Donoghue, D.J., Hobbs, J.E.,ORA, FDA, Atlanta GA and University of Arkansas Fayetteville AR (2003). Determination of N-Methyl Carbamate Pesticide Residue in Eggs at PPB Levels using a Solid Phase Extraction Cleanup. 2003 FDA Science Forum Poster Abstract: 299.

[11] (*) denotes that the maximum residue limit (MRL) has been set "at or about" the limit of analytical quantitation.

Summary of trials considered as part of the review of carbaryl.

crop group	crop	PHI	application rate	highest residue	median residue	number of trials for crop	number of trials for whole group
FB	blackberries	2 to 3	0.1 kg ai/hL	4.4	NA	3	
FB	blueberries	3	0.1 kg ai/hL	1.02	NA	1	
FB	cranberries	3	0.1 kg ai/hL	1.01	NA	2	
FB	grapes	3 to 4	0.1 kg ai/hL	5.1	1.275	16	
FB	raspberries	2 to 4	0.1 kg ai/hL	14.6	4.34	9	
FB	strawberries	3 to 4	0.1 kg ai/hL	1.88	NA	2	33
FC	grapefruit	5	0.48 kg ai/hL	6.8	NA	1	
FC	lemon	5	0.48 kg ai/hL	5.1	1.94	3	
FC	orange	2 to 5	0.48 kg ai/hL	8.1	3.8	9	13
FI	avocado	3	0.1 kg ai/hL	0.57	NA	1	
FI	persimmon	3 to 4	0.1 kg ai/hL	2.3	NA	3	4
FP	apple (fruit thinning use)	78 to 82	0.1 kg ai/hL	0	0	5	
FP	apple (late pre-harvest appl)	2 to 4	0.1 kg ai/hL	5.75	0.605	16	
FP	pear	2 to 3	0.1 kg ai/hL	1.84	0.25	5	26
FS	apricot	3	0.1 kg ai/hL	1.77	NA	2	
FS	cherry	3	0.1 kg ai/hL	4.6	1.725	8	
FS	peach	3	0.1 kg ai/hL	2.14	0.86	13	
FS	plum	3	0.1 kg ai/hL	0.61	0.275	6	29
FT	olives	0	0.1 kg ai/hL	0.89	NA	2	2
SO	cotton	0 to 3	1.1 kg ai/ha	0.87	0.485	5	5
TN	almonds	0	0.1 kg ai/hL, 1.1 kg ai/ha	0.88	0.52	7	7
VA	onion	3	1.1 kg ai/ha	1.19	NA	1	1
VB	broccoli	3 to 4	1.1 kg ai/ha	3.96	1.93	13	
VB	Brussels sprouts	3 to 4	1.1 kg ai/ha	2.01	NA	2	
VB	cabbage	2 to 4	0.1 kg ai/hL, 1.1 kg ai/ha	15.8	0.925	44	
VB	cauliflower	3 to 4	0.1 kg ai/hL, 1.1 kg ai/ha	26.59	7.6	15	
VB	kohlrabi	3 to 4	1.1 kg ai/ha	10	NA	2	76
VC	cantaloupe	3	1.1 kg ai/ha	1.49	0.69	5	
VC	Cucumber	2 to 3	1.1 kg ai/ha	1.9	0.8	5	
VC	gourds (ridge, sponge, bottle)	2 to 3	0.1 kg ai/hL, 1.1 kg ai/ha	14.85	0.06	8	
VC	long melon	3	0.1 kg ai/hL	6	NA	1	
VC	pumpkin (winter squash)	3	1.1 kg ai/ha	0.39	NA	2	
VC	zucchini (summer squash)	2 to 3	1.1 kg ai/ha	1.29	0.27	9	38
VD	blackeyed bean	3	1.1 kg ai/ha	2.21	NA	1	
VD	cowpea	3	1.1 kg ai/ha	0.75	0.27	4	5
VL	beet tops	2 to 3	1.1 kg ai/ha	7	NA	2	
VL	Chinese cabbage	3 to 4	1.1 kg ai/ha	19.2	2.7	6	
VL	Collards (kale)	2 to 3	1.1 kg ai/ha	21.5	1.87	10	

VL	endive	2 to 3	1.1 kg ai/ha	15.5	10.1	7	
VL	lettuce, head	2 to 4	1.1 kg ai/ha	7.17	1.87	14	
VL	lettuce, leaf	2 to 3	1.1 kg ai/ha	27	NA	2	
VL	mustard greens	2 to 3	1.1 kg ai/ha	31.2	20	3	
VL	spinach	2 to 4	0.1 kg ai/hL, 1.1 kg ai/ha	24.5	8.855	8	
VL	Swiss chard	2 to 3	1.1 kg ai/ha	30.1	6	3	
VL	turnip tops	2 to 3	1.1 kg ai/ha	21	NA	3	58
VO	capsicums	3	0.1 kg ai/hL, 1.1 kg ai/ha	1.93	0.8	17	
VO	chilli	2 to 3	1.1 kg ai/ha	19	6.17	10	
VO	eggplant	2 to 4	0.1 kg ai/hL, 1.1 kg ai/ha	9	0.575	33	
VO	okra	2 to 4	0.1 kg ai/hL, 1.1 kg ai/ha	9.6	0.72	32	
VO	sweetcorn	2 to 4	1.1 kg ai/ha	3.04	0.2	23	
VO	tomato	2 to 4	0.1 kg ai/hL, 1.1 kg ai/ha	16.3	0.5	56	171
VP	beans (green, succulent)	2 to 4	1.1 kg ai/ha	4	1.06	17	
VP	pea (garden, field, succulent)	2 to 4	1.1 kg ai/ha	4.24	0.79	23	42
VR	carrots	4	1.1 kg ai/ha	0.06	NA	1	
VR	garden beet	3	1.1 kg ai/ha	0.14	NA	1	
VR	potato	2 to 4	1.1 kg ai/ha	0.06	0.01	9	
VR	sugarbeet	3 to 4	1.1 kg ai/ha	0.24	0	8	
VR	turnip	3	1.1 kg ai/ha	0.65	0.45	5	24
VS	asparagus	2 to 3	1.1 kg ai/ha	3.4	0.475	6	
VS	celery	3	1.1 kg ai/ha	5.1	2.3	3	9

2.5.1 Residues in berry fruit

Various Berry Crops - 33 residues trials where the use patterns were considered relevant to the approved Australian uses (PHI 3±1 days) were available for review.

Grapes - 17 trials on gave highest residues of 5.1 mg/kg with median residues of 1.275 mg/kg. The data support an MRL of 7 mg/kg for grapes.

Raspberries - 9 trials were available. Highest residues in the trials were 14.6 mg/kg, with median residues of 4.34 mg/kg. The data support an MRL of 20 mg/kg for raspberries.

Blackberries - 3 trials were available, where highest residues were 4.4 mg/kg. Relevant data were limited for other berry crops. Only a single residues trial was available for blueberries, while two trials were available for cranberries and strawberries. Insufficient residues data are available to establish an MRL for blackberries, cranberries, strawberries or blueberries.

2.5.2 Residues in citrus fruit

Citrus crops - 13 residues trials were provide. Where the use patterns were considered relevant to the approved Australian uses (PHI 2-5 days), were available for review.

Oranges - 9 relevant trials were provided. Highest residues in the trials were 8.1 mg/kg, with a median residue of 3.8 mg/kg. The data support an MRL of 10 mg/kg for oranges.

There were limited data available for other citrus fruits. A single trial was provided for grapefruit (HR 6.8 mg/kg) and three trials on lemons (HR 5.1 mg/kg). No relevant data were available for other citrus fruits.

There were insufficient data provided to support separate MRLs for grapefruit, lemons or other citrus fruits, and there are insufficient residues data available to establish a group MRL for citrus fruits.

2.5.3 Residues in pome fruit

Registered products containing carbaryl may be used on pome fruit as a foliar spray for control of insect pests, and as a foliar spray several months before harvest to aid fruit thinning. The fruit thinning use pattern involves a single application between 7-28 days after full bloom. The current Withholding Period (WHP) for this use is 3 days, however the actual time between application and harvest would be much longer. The period between the end of flowering and harvest for most apple varieties in Australia is approximately 4 months, therefore it is estimated that application 7-28 days after full bloom would be at least 3 months before harvest.

Data assessed

A total of 26 relevant trials were available for pome fruit, 16 for the use involving a late pre-harvest application and 5 for the fruit thinning use pattern, where application occurs several months before harvest.

Apples - 16 trials gave maximum residues of 5.75 mg/kg and a median residue of 0.605 mg/kg (PHI 3±1 days). Five (5) relevant trials were provided for pears. Maximum residues in these trials were 1.84 mg/kg with a median residue of 0.25 mg/kg (PHI 3±1 days). The data on apples and pears support a group MRL of 10 mg/kg for pome fruit for the late pre-harvest use pattern.

In 5 trials on apples, application almost 3 months before harvest resulted in residues in fruit of less than 0.01 mg/kg. In 2 trials on pears approximately 2 months before harvest, residues in fruit at harvest were non-detectable. At a WHP of approximately 90 days residues in fruit are expected to be low. The data support an MRL of 0.01 mg/kg for pome fruit for use of carbaryl as an aid to fruit thinning.

Further trial data submitted

Determination of carbaryl residues in apples and pears. Burn, R (2005). Serve-Ag Research Pty Ltd Report No AP03002.

Current withholding period = 3 days
Current MRL = 5 mg/kg
Label treatment rates = 80-100g ai/100L for early fruit caterpillars, codling and light-brown apple moths, blister mite, pear and cherry slug and fruit thinning

Details of the trials undertaken are recorded in the table below.

Trial details of treatment of apple and pear trees with the carbaryl product Bugmaster®

Crop (Location)	No of Trials	Dates	No of applic^{n}s (interval)	Rate per application (g ai/hL)	Volume (L/ha)	Stage of growth at final applicn	Stage of growth at sampling	Method of application
Pears (SA)	1	04/05	3 (14-15 days)	100	1200-1800	5 cm	Mature	Motorised hand lance
Apples (Tas)	1	04/05	3 (10-11 days)	100	1600	2.5-3 cm	Mature	Pressurised sprayer
Pears (Vic)	1	04/05	3 (13-14 days)	100	1027-1347	3-9 cm	Ripe fruit	Backpack mister

Results of analyses carried out on the samples are shown in the following table.

Table 48: Residues of carbaryl in pome fruit following application of Bugmaster®.

Commodity	Location	Final application rate* (g ai/100L)	Final volume** (L/ha)	Portion analysed	PHI (days)	Residue (mg/kg)
Pear	SA	100	1800	Whole	71	<0.04
Apple	Tas	100	1600	Whole	110	<0.04
Pear	Vic	100	1027-1167	Whole	56	0.34
					63	0.16
					70	0.12
					77	0.09

Some data were previously considered in the 2005 report. These are reproduced in the following table.

Residues of carbaryl in pome fruit following application of Bugmaster® - previous data.

Commodity	Location	Final application rate* (g ai/100L)	Final volume** (L/ha)	Portion analysed	PHI (days)	Residue (mg/kg)
Apples	QLD	100	3100-4500	Whole	35	1.04
					49	0.47
					63	0.24
					77	0.14

Discussion of results

Acute dietary intake data indicated that a maximum residue in fruit of 0.29 mg/kg carbaryl was appropriate, and this was equivalent to 98% of the ARfD.

It was proposed previously to set the MRL at 0.2 mg/kg. When this figure is substituted in the NESTI calculation for 2-6 year-olds, the portion of the ARfD for apples, pears and loquats is 67, 83 and 35%, respectively, which are all acceptable.

The residues data indicate that residues at 63 days would be 0.16-0.24 mg/kg, from two samples. Residues at 70 and 77 days PHI (by interpolation where necessary) would be 0.12-0.19 mg/kg and 0.09-0.14 mg/kg respectively. A separate result in apples at 71 days was <0.04 mg/kg, well below the proposed MRL.

The evaluator is not satisfied that the use of carbaryl can be restricted to fruit below a certain size, or that size at time of treatment means anything relative to the final harvestable commodity, because of variety differences in development. The use of a withholding period is considered more practical. In consultation with the industry (Kevin Bodnaruk – personal communication), the preference of growers is that they would prefer to work with a withholding period, rather than a stage of growth restricting when they could apply carbaryl.

The NRS has monitored the presence of carbaryl in apples and pears for many years. The data on its web site[12[1]] indicate that carbaryl residues are found in about 3.5 % of samples every year, with the limit of reporting being 0.1 mg/kg. The data further indicate that the proposed MRL of 0.2 mg/kg would be exceeded in about half of those samples with residues, if the present use pattern was maintained. Therefore, some changes to the use pattern of carbaryl in pome fruit would be required, with uses being restricted to the period prior to 77 days pre-harvest. The use for caterpillars, moths, slugs and mites would only be possible in the early development of the tree, otherwise residues could exceed the MRL.

Allowing for the low confidence in residues complying with an MRL of 0.2 mg/kg, because of the small number of data in support of any time point, a harvest-withholding period of 77 days is recommended.

2.5.4 Residues in stone fruit

Stone fruit - 29 residues trials on, where the use patterns were considered relevant to the approved Australian uses (PHI 3 days), were provided for review.

Peaches - 13 trials on gave highest residues of 2.14 mg/kg with a median residue of 0.86 mg/kg (PHI 3 days). The data support an MRL for peaches of 5 mg/kg.

Cherries - 8 trials on gave highest residues of 4.6 mg/kg and a median residue of 1.725 mg/kg (PHI 3 days). The data support an MRL for cherries of 10 mg/kg.

Plums - 6 trials gave highest residues of 0.61 mg/kg and a median residue of 0.275 mg/kg (PHI 3 days). The data support an MRL for plums of 3 mg/kg.

Apricots - Only 2 relevant trials were available and no data were available for nectarines. The data on apricots are insufficient to establish an appropriate MRL for this commodity. However, based on the similar size and skin texture of apricots and nectarines to plums, the data on plums could be used to set MRLs for apricots and nectarines. MRLs of 3 mg/kg for apricots and nectarines are appropriate.

2.5.5 Residues in tropical fruit

Tropical fruit - 6 relevant trials where provide, where the use patterns were considered similar to the approved Australian uses, were available for review. Three (3) trials on persimmons gave maximum residues of 2.3 mg/kg while a single trial on avocado gave residues of 0.57 mg/kg (PHI 3-4 days). Two (2) trials on olives gave maximum residues of 0.89 mg/kg (PHI 0 days).

[12[1]] http://www.affa.gov.au/content/output.cfm?ObjectID=BFB97D42-5A89-4755-8DCE8352CA4EDEB8

There are insufficient residues data available to support MRLs for individual tropical fruits or a group MRL.

2.5.6 Residues in bulb vegetables

Only a single relevant residues trial was available for bulb vegetables. In that trial, residues of 1.19 mg/kg occurred in onions (PHI 3 days).

There are insufficient residues data available to establish an MRL for bulb vegetables.

2.5.7 Residues in brassica vegetables

Brassica Vegetables - 76 relevant residues trials were available.

Forty-four (44) trials were available for cabbage, where highest residues of 15.8 mg/kg and median residues of 0.925 mg/kg occurred in treated crops (PHI 2-4 days). Only 4 of the trials gave residues in cabbage above 10 mg/kg. The data support an MRL of 15 mg/kg for cabbage.

Cauliflower - 15 trials were available, where highest residues of 26.6 mg/kg and median residues of 7.6 mg/kg occurred in treated crops (PHI 3-4 days). Only a single trial gave residues in cauliflower above 20 mg/kg. The next highest residue in cauliflower was 18.4 mg/kg. The data support an MRL of 20 mg/kg for cauliflower.

Broccoli - 13 trials available for gave highest residues of 3.96 mg/kg and median residues of 1.93 mg/kg in treated crops (PHI 3-4 days). The data support an MRL of 7 mg/kg for broccoli.

Brussels Sprouts - 2 trials on (HR 2.01 mg/kg, PHI 3-4 days) and two trials on kohlrabi (HR 10 mg/kg, PHI 3-4 days) were available. The data are insufficient to establish separate MRLs for these commodities. No relevant trials were available for other brassica vegetables.

2.5.8 Residues in cucurbit vegetables

Cucurbit Vegetables - 38 relevant trials were available.

Cucumber - 5 trials gave highest residues of 1.9 mg/kg and median residues of 0.8 mg/kg (PHI 2-3 days). The data support an MRL of 5 mg/kg for cucumber.

Cantaloupe (rock melon) - 5 trials gave highest residues of 1.9 mg/kg and median residues of 0.69 mg/kg. The data support an MRL of 3 mg/kg for cantaloupe.

Summer squash (zucchini) - 9 trials gave highest residues of 1.29 mg/kg and median residues of 0.27 mg/kg. The data support an MRL for summer squash of 3 mg/kg.

Residues trials were available for several types of gourd: Six (6) trials on bottle gourd (HR 0.09 mg/kg), 1 trial on ridge gourd (HR 8.02 mg/kg) and 1 trial on sponge gourd (14.85 mg/kg) (PHI 2-3 days). The data support an MRL of 0.2 mg/kg for bottle gourd but are insufficient to allow the establishment of MRLs for other gourds.

Only 2 trials on winter squash (pumpkin) and one trial on long melon were available. The data are insufficient to allow the establishment of a separate MRL for pumpkins or long melons.

2.5.9 Residues in leafy vegetables

Leafy vegetables - 58 relevant residues trials were available.

Highest residues occurring in various leafy crops were similar. Highest residues ranged from 15.5 mg/kg up to 31.2 mg/kg in trials on Chinese cabbage (6 trials), collards/kale (10 trials), endive (7 trials), leaf lettuce (2 trials), mustard greens (3 trials), spinach (8 trials), turnip tops (3 trials) and Swiss chard (3 trials). Median residues in these trials were 9.075 mg/kg (PHI 2-4 days). Only 2 samples contained residues exceeding 30 mg/kg, one in Swiss chard and one in mustard greens.

Residues occurring in head lettuce and beet tops were lower than other leafy vegetables, with highest residues of 7.17 mg/kg and median residues of 1.87 mg/kg occurring in 14 trials on head lettuce (PHI 2-4 days) and maximum residues in beet tops from 2 trials of 7.0 mg/kg.

The data support a group MRL of 30 mg/kg for leafy vegetables (except head lettuce) and an MRL of 10 mg/kg for head lettuce.

2.5.10 Residues in fruiting vegetables

Results from a total of 171 relevant residues trials were available for fruiting vegetables (non-cucurbits).

Chilli – 10 trials gave highest residues of 19 mg/kg with a median residue of 6.17 mg/kg. The next highest result was 12.9 mg/kg. The data support an MRL of 20 mg/kg for chilli peppers.

Capsicums - 17 trials gave highest residues of 1.93 mg/kg with a median residue of 0.8 mg/kg. The data support an MRL of 3 mg/kg for capsicums.

Eggplant - 33 trials gave highest residues of 9 mg/kg with a median residue of 0.575 mg/kg. The next highest result was 5.29 mg/kg, and residues in eggplant exceeded 3 mg/kg in only four samples. The sample containing residues of 9 mg/kg may be considered an outlier. The data support an MRL of 7 mg/kg for eggplant.

Okra - 32 trials gave highest residues of 9.6 mg/kg with a median residue of 0.72 mg/kg. Of the 32 samples, 30 contained residues below 3.2 mg/kg. The remaining 2 samples contained residues of 9.2 and 9.6 mg/kg. Given the large number of trials, the 2 results above 9 mg/kg may be considered outliers. The data support an MRL of 5 mg/kg for okra.

Sweet corn - 23 trials gave highest residues of 3.04 mg/kg with a median residue of 0.2 mg/kg. The next highest residue in the trials was 1.34 mg/kg. The sample containing residues of 3.04 mg/kg may be considered an outlier. The data support an MRL of 3 mg/kg for sweet corn.

Tomatoes - 56 trials gave a highest residue of 16.3 mg/kg, with a median residue of 0.535 mg/kg. Two (2) samples contained residues of 13.6 and 16.3 mg/kg, while the remainder of the samples in the trials contained residues of less than 8.9 mg/kg. Given the large number of trials for tomatoes, the two samples containing highest residues may be considered to be outliers. The data support an MRL of 10 mg/kg for tomatoes.

No relevant residues data were available for mushrooms or Cape gooseberries to allow the establishment of suitable MRLs.

2.5.11 Residues in pulses

Four relevant residues trials were available for pulses: three (3) trials on cowpeas gave highest residues of 0.75 mg/kg and 1 trial on black eyed bean gave residues of 2.21 mg/kg (PHI 3 days). The data are insufficient to allow the establishment of suitable MRLs for pulses.

2.5.12 Residues in legumes

Legume vegetables - 42 relevant residues trials were available.

Green beans - 17 trials were available, highest residues of 4 mg/kg occurred in treated produce, with a median residue of 1.06 mg/kg (PHI 2-4 days). Twenty-three (23) trials on peas gave highest residues of 4.24 mg/kg, with a median residue of 0.79 mg/kg. The data available for peas and beans support a group MRL of 7 mg/kg for legume vegetables.

2.5.13 Residues in root and tuber vegetables

Root and tuber vegetables - 24 relevant residues trials were available, where the use patterns were considered similar to Australian uses.

Potatoes - 9 trials gave highest residues of 0.06 mg/kg, with a median residue of 0.01 mg/kg (PHI 2-4 days). The data support an MRL of 0.1 mg/kg for potatoes.

Sugar beets - 8 trials gave a highest residue of 0.24 mg/kg, with a median residue of <0.02 mg/kg (PHI 3-4 days). A single trial on garden beet gave a residue of 0.14 mg/kg (PHI 3 days). The data support an MRL of 0.5 mg/kg for sugar beet. Sugar beets may be considered similar to beetroot therefore extrapolation of these data allows the establishment of an MRL of 0.5 mg/kg for beetroot.

Turnips (Swede) - 5 trials gave a highest residue of 0.65 mg/kg, and a median residue of 0.45 mg/kg. The data support an MRL of 2 mg/kg for Swede.

There were no suitable residues data available for carrots and parsnips to allow the establishment of a suitable MRL for these commodities.

2.5.14 Residues in stalk and stem vegetables

Stalk and stem vegetables - 9 relevant residues trials were available.

Asparagus - 6 trials resulted in highest residues of 3.4 mg/kg, with a median residue of 0.475 mg/kg. The data support an MRL of 5 mg/kg for asparagus.

Celery - 3 trials resulted in a highest residue of 5.1 mg/kg. The data are insufficient to allow the establishment of an MRL for celery. There are insufficient data available to establish an MRL for other stalk and stem vegetables.

2.6 RESIDUES IN OILSEEDS

Registered carbaryl products may be used on cotton, sunflower and linseed crops.

Oilseeds - 5 residues trials, where the use patterns were considered relevant to the approved Australian uses, were available for review.

Cottonseed - 5 trials were available where treatment occurred 0-3 days before harvest. Highest residues of 0.87 mg/kg occurred in seed, with a median residue of 0.485 mg/kg. The data support an MRL for cottonseed of 3 mg/kg.

There were no relevant residues trial data for other oilseed crops therefore no MRLs can be established for sunflower or linseed.

2.7 RESIDUES IN TREE NUTS

Registered carbaryl products may be used on macadamia nuts and pecan nuts. Seven (7) residues trials on almonds, where the use patterns were considered relevant to the approved Australian uses, were available for review.

In the 7 trials on almonds highest residues of 0.88 mg/kg occurred in nuts, with median residues of 0.52 mg/kg (PHI 0 days). No suitable residues data were available for macadamia or pecan nuts. The data on almonds may be used to establish an MRL for macadamia nuts and pecan nuts and 2 mg/kg.

2.8 EFFECT OF WASHING AND COOKING ON RESIDUES IN FRUITS AND VEGETABLES

The effect of washing and cooking on residue levels in various commodities was examined in some studies. The level of carbaryl residues was found to decrease by an average of approximately 80% after washing and 80% after cooking. Between 84-100% of residues in cowpea whole pods were removed by cooking.

In several trials on citrus fruits residues were determined separately in skin and pulp, with residues in the whole fruit calculated from these values. The majority of residues were found to be present in peel, and on average residues in the citrus pulp were 0.05 times the level in the whole fruit.

In trials on bananas treated with carbaryl, residues were determined separately in peel and pulp. Residues in pulp and peel were found to be similar in magnitude, with the average ratio of residues in pulp to peel of 0.92 from 17 samples.

2.9 DIETARY EXPOSURE

In December 2002, the Acceptable Daily Intake (ADI) for carbaryl was increased from 0.004 to 0.008 mg/kg bw/day and an acute reference dose (ARfD) was set at 0.01 mg/kg bw.[13] The APVMA was advised to assess whether the current use patterns appearing on registered product labels would result in dietary exposure that would exceed the revised ADI for lifetime exposure

[13] The OCS recommended a new ARfD and revised the ADI for carbaryl in its report dated December 2002.

(chronic dietary intake) or the ARfD for short term exposure (acute dietary exposure).

2.9.1 Short-term dietary exposure

Acute or short term dietary exposure to pesticide residues is estimated using procedures and methodology recommended by the joint consultation of the WHO and FAO using a National NESTI calculation. The calculations utilise commodity unit weights (Bowles and Hamilton, 2001), 97.5th percentile dietary consumption figures (ie large portion sizes) from the 1995 Australian National Nutrition Survey (1995) and results from relevant residues trials.

Where insufficient residue trial data were available, the highest residue (HR) from trials of a similar crop or the current MRL was used as the HR value in the NESTI calculations. A minimum of 41 consumers is required in the dietary survey results to adequately determine the 97.5th percentile consumption figure. Where the number of consumers was less than 41, large portion sizes of similar commodities were used. Where the number of consumers was still <41, the consumption figure for the entire crop group was used as a conservative estimate.

The full NESTI calculations for children aged 2-6 years and for the general population aged 2 years and above are summarised in the the following table.

NESTIs (as % of ARfD) for children aged 2-6 years and general population aged >2 years.

Crop group	Crop	NESTI 2-6 years	NESTI >2 years	Commodities where NESTI <ARfD
FB	**berry fruits**			
	blueberries	184	33	
	blackberries	792	143	
	dewberries (incl boysen, logan)	918	166	
	gooseberry	918	166	
	grapes	2059	736	
	raspberries	37	21	raspberries
	strawberries	178	55	
FC	**citrus fruits**			
	mandarins	108	30	
	oranges	138	42	oranges
	grapefruit	160	44	
	lemons	118	9	lemons
FI	**tropical fruit (inedible peel)**			
	jambu	2141	531	
	avocado	296	49	
	banana (in pulp)	1159	319	
	custard apple	3562	934	
	elephant apple	2930	755	
	feijoa	2299	576	
	jackfruit	5001	1191	
	kiwifruit	4134	454	
	litchi	1667	397	
	longan	1667	397	
	mango	2960	1000	

	papaya	4438	1183	
	granadilla	1667	397	
	passionfruit	1667	196	
	rambutan	1667	397	
	sapodilla	2299	576	
	sapote	3439	899	
FP	**pome fruit**			
	apples	1929	626	
	pears	760	201	
	loquats	1005	364	
	apples (fruit thinning use)	0	0	apples
	pears (fruit thinning use)	0	0	pears
	loquats (fruit thinning use)	0	0	loquats (fruit thinning use)
FS	**stone fruit**			
	cherries	786	96	
	plums (including prunes)	159	58	plums (including prunes)
	apricot	491	179	apricot
	nectarine	789	335	nectarine
	peach	649	252	peach
FT	**tropical fruit (edible peel)**			
	carambola	0	743	
	grumichama (Brazillian cherry)	0	515	
	jaboticaba	0	515	
	olives	0	11	olives
	guava	0	674	
MM	**animal commodities**			
	mammalian offal	2	6	mammalian offal
	meat mammalian	3	2	meat mammalian
	milks	12	3	milks
	poultry offal	4	1	poultry offal
	poultry meat	2	1	poultry meat
	eggs	1	0	eggs
TN	**tree nuts**			
	macadamias	13	1	macadamias
	pecans	10	3	pecans
SO	**oilseeds**			
	sunflower seed	4	2	
	cotton seed	4	2	cotton seed
	linseed	4	2	
VB	**brassica vegetables**			
	cabbage	2065	1539	
	broccoli	1045	334	
	Brussels sprouts	160	70	
	cauliflower	5542	2096	
VC	**cucurbit vegetables**			
	cucumber	306	109	
	pumpkin	138	45	
	zucchini	226	58	
	watermelon	1819	1853	

	cantaloupe (rock melon)	903	257	
VL	**leafy vegetables**			
	mizuna	1759	1115	
	chard	2757	1853	
	chervil	1759	1115	
	chinese cabbage	1759	2268	
	lettuce, head	825	372	
	lettuce, leaf	3106	1801	
	rucola (rocket)	1759	1115	
	spinach	1607	1633	
VO	**fruiting vegetables**			
	Eggplant	1407	1055	
	okra	284	167	
	capsicums	183	79	
	chilli peppers	600	73	
	sweetcorn (corn on the cob)	375	102	
	tomato	3641	1236	
	mushrooms	139	83	
VR	**root vegetables**			
	potato	18	7	potato
	carrot	123	25	
	turnip	32	18	turnip
	beetroot	12	11	beetroot
VP	**legume vegetables**			
	beans	203	84	
	peas	179	101	
VD	**pulse vegetables**			
VA	**bulb vegetables**			
	onions	120	56	
	leek	504	270	
VS	**stalk and stem vegetables**			
	celery	674	202	
	asparagus	149	122	
CF	**Cereals**			
	wheat bran, unprocessed	23	39	wheat bran, unprocessed

The above calculations and discussion on acute dietary risk has highlighted that, with the exception of the commodities listed above, the use of registered products containing carbaryl in accordance with approved labels may present an acute dietary risk to consumers of treated produce.

2.9.2 Long-term dietary exposure

Carbaryl has not been included in any of the Australia and New Zealand Food Authority (ANZFA) Market Basket Surveys or Total Diet Surveys of the last decade and so there is no information on actual dietary exposure. In such cases conservative models that overestimate chronic dietary intake are used to establish human safety. The model used for chronic dietary exposure to pesticides in Australia and recommended by the joint consultation of the WHO and FAO is the National Estimated Dietary Intake (NEDI) calculation. In this calculation use is

made of survey results for agricultural commodities, processing factors for commodities such as washing, peeling or cooking, and median or maximum residues for "worst-case" in available residues trials.

Survey data for carbaryl residues in different commodities were obtained from the NRS and are tabulated in the following table.

Survey data for carbaryl in raw agricultural commodities (data for 1995-1999 except apples and pears which include data from 01/1/99-25/2/2000).

Commodity	Number samples tested	Number of detections	Comment on detection residue levels
Macadamia & pecan nuts	378	0	-
Barley	1317	52	32 <0.1 mg/kg, 20<1 mg/kg
Wheat bran	209	33	18 <0.1 mg/kg, 15 <4 mg/kg
Canola	254	15	15<0.1 mg/kg
Faba beans	2	0	-
Wheat flour	209	0	-
Lupins	346	2	1<0.1 mg/kg, 1<0.2 mg/kg
Oats	116	8	5<0.1 mg/kg, 3<1 mg/kg
Peas (inc field & chick peas)	212	3	3<0.3 mg/kg
Sorghum	642	40	33<0.1 ppm, 6<1 mg/kg, 1<5 mg/kg
Triticale	1	0	-
Wheat	5800	234	186<0.1 mg/kg, 45<1 mg/kg, 3<2.5 mg/kg
Apples	205	7	5<0.05 mg/kg, 2<1 mg/kg
Pears	8	0	-
Grapes	39	1	1<2.5 mg/kg
Stone fruit	20	2	2 <5 mg/kg
Tomato	20	0	-

The NEDI calculation was based on the uses for which there were sufficient residues data to establish an MRL and where use of registered products according to label directions would not result in short term dietary exposure exceeding the acute reference dose.

The chronic dietary exposure to carbaryl is calculated at approximately 4% of the acceptable daily intake. Since the NEDI calculation is less than the ADI it is concluded that the chronic or long term dietary exposure to carbaryl residues should not present an undue risk to the health of consumers of treated produce when registered products, containing the label amendments described above, are used as directed.

2.10 NON-FOOD USES

Registered carbaryl products may be used in a variety of non-food (human or livestock) situations: as an insecticide in commercial, industrial and domestic areas, tobacco storage sheds and rights of way, in non-crop areas in general, ornamentals, lawns, elm trees (in non-crop areas), kenaf, Duboisia and rosella, and for disinfestation of grain storage buildings.

Table 5 of the *MRL Standard* refers to uses of substances where maximum residue limits are not necessary. Specifically, these include situations where residues do not or should not occur in foods or animal feeds; or where the residues are identical to or indistinguishable from natural food components; or are otherwise of no toxicological significance.

Table 5 *MRL Standard* entries are required for non-food use patterns appearing on all registered product labels. The following Table 5 *MRL Standard* entries are recommended:

Table 5 *MRL Standard* entries

Substance	Use
ADD: Carbaryl	• As an insecticide in non-crop areas including commercial, industrial and domestic areas, tobacco storage sheds and rights of way • As an insecticide on ornamentals and other non-food or animal feed crops and trees • For the disinfestation of grain storage buildings • On tropical fruits, prior to flowering, and when fruit are not on the tree • For control of cutworm on grapes, when applied to the base of the vine only • On cucurbits/melons, prior to commencement of flowering • On avocados, prior to flowering, and when fruit are not on the tree • On mangoes, prior to flowering, and when fruit are not on the tree

2.11 OTHER CONSIDERATIONS

In addition to uses on registered product labels, carbaryl may be used under permit in various situations including several in-crop uses. The APVMA's powers in relation to permits are covered by Part 7, Sections 108-119 of the AgVet Chemicals Code Act. Therefore, current carbaryl permits fall outside the scope of the review.

However, some uses permitted under current permits are covered by existing MRLs. Suitable MRLs must remain in place while the permit is current. The current MRLs which cover uses approved under permit, and which are recommended for deletion or amendment as part of this review, are as follows:

Table 1 *MRL Standard* entries

Compound		Food	MRL (mg/kg)
Carbaryl			
DELETE:	FS 0240	Apricot	10
	VS 0621	Asparagus	10
	FI 0326	Avocado	10
	FI 0327	Banana [in the pulp]	5
	FB 0264	Blackberries	10
	FB 0020	Blueberries	7
	FT 0289	Carambola	5
	GC 0080	Cereal grains	T5
	FS 0013	Cherries	5
	FC 0001	Citrus fruits	7
	SO 0691	Cotton seed	1
	FI 0332	Custard apple	5
	FB 0266	Dewberries (including Boysenberry and Loganberry)	10
	MO 0105	Edible offal (mammalian)	T0.2
	PE 0112	Eggs	T0.2
	FI 0371	Elephant apple	5
	FI 0335	Feijoa	5

Compound		Food	MRL (mg/kg)
	VC 0045	Fruiting vegetables, Cucurbits	3
	FI 0351	Granadilla	5
	FB 0269	Grapes	5
	FT 0298	Grumichama [Brazilian cherry]	5
	FT 0336	Guava	5
	FT 0300	Jaboticaba	5
	FI 0338	Jackfruit	5
		Jambu	5
	FI 0341	Kiwifruit	10
	VL 0053	Leafy vegetables	10
	FI 0343	Litchi	5
	FI 0342	Longan	5
	FI 0345	Mango	5
	MM 0095	Meat [mammalian]	T0.2
	ML 0106	Milks	T*0.05
	FS 0245	Nectarine	10
	VO 0442	Okra	10
	FT 0305	Olives	10
	DM 0305	Olives, processed	1
	FI 0350	Papaya [pawpaw]	5
	FI 0351	Passion fruit	5
	FS 0247	Peach	10
	FS 0014	Plums (including Prunes)	5
	FP 0009	Pome fruits	5
	VR 0589	Potato	0.2
	PO 0111	Poultry, Edible offal of	T5
	PM 0110	Poultry meat	T0.5
	FI 0358	Rambutan	5
	FB 0272	Raspberries	10
	FI 0359	Sapodilla	5
	FI 0360	Sapote, Black	5
	FI 0361	Sapote, Green	5
	FI 0362	Sapote, Mammey	5
	FI 0363	Sapote, White [casimiroa]	5
	FB 0275	Strawberry	7
	GS 0659	Sugar cane	T*0.05
	SO 0702	Sunflower seed	1
	VO 0447	Sweet corn (corn-on-the-cob)	1
	TN 0085	Tree nuts	1
	TN 0085	Tree nuts [whole in shell]	10
		Vegetables [except asparagus; fruiting vegetables, cucurbits; leafy vegetables; okra; potato; sweet corn (corn-on-the-cob)]	5
	CM 0654	Wheat bran, unprocessed	T20
ADD:	VR 0574	Beetroot	0.5
	GC 0080	Cereal grains	15
	SO 0691	Cotton seed	3
	MO 0105	Edible offal (mammalian)	0.2

Compound		Food	MRL (mg/kg)
	PE 0112	Eggs	*0.02
	FC 0204	Lemon	7
	TN 0669	Macadamia nut	2
	MM 0095	Meat [mammalian]	*0.02
	ML 0106	Milks	*0.02
	FC 0004	Oranges, Sweet, Sour	7
	TN 0672	Pecan	2
	FP 0009	Pome fruits	0.2
	VR 0589	Potato	0.1
	PM 0110	Poultry meat	*0.02
	PO 0111	Poultry, Edible offal of	0.2
	FB 0272	Raspberries, Red, Black	20
	FS 0012	Stone fruits [except cherry]	0.5
	VR 0596	Sugarbeet	0.5
	VR 0497	Swede	2
	CM 0654	Wheat bran, unprocessed	30

Table 4 *MRL Standard* entries

Compound		Animal feed commodity	MRL (mg/kg)
Carbaryl			
DELETE:	AF 0080	Forage of cereal grains	T100
	AS 0081	Straw and fodder (dry) of cereal grains	T100
ADD:		Cereal forage (green)	100
		Grass pastures (green)	400
	AS 0162	Hay or fodder (dry) of grasses	300
		Legume forage (green)	400
		Legume fodder	100
	AM 0165	Miscellaneous fodder and forage crops	300
		Sorghum bran	50
	AS 0081	Straw and fodder (dry) of cereal grains	100

An increase in the current raspberry MRL from 10 to 20 mg/kg was recommended as part of this review therefore the new MRL will cover residues occurring as a result of the permit use pattern. An increase in the cereal grain MRL from 5 to 15 mg/kg, and establishment of permanent entries for cereal forage, straw and fodder in Table 4 of the *MRL Standard*, were also recommended. These new MRLs will cover residues occurring in maize treated under the current permit.

Uses of carbaryl approved under permit should be considered before any changes are made to the MRLs for the commodities listed above.

2.11.1 Trade

Use patterns appearing on approved product labels of registered carbaryl products will not change as a result of this review. As a consequence, the situation with regards to residues-in-trade issues is unchanged from the present situation.

2.12 REFERENCES

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Appendix 1, Current entries in the *MRL Standard* (February 2000) and comparison with international MRLs

Table 1:

Food Commodity	Australia	CODEX	USA	Canada	France	Germany	EU	Japan	Korea	Malaysia	Singapore	Taiwan
FS 0240 Apricot	10	10	10	10	3	3	3		1		10	1
VS 0621 Asparagus	10	10	10	10	1	1	1		1	4	10	
FI 0326 Avocado	10				1	1	1		1		10	0.1
FI 0327 Banana [in the pulp]	5	5	10		1	1	1					0.1
FB 0264 Blackberries	10	10	12	10	1	1	1				10	0.5
FB 0020 Blueberries	7	7	10	7	1	1	1				7	0.5
FT 0289 Carambola	5				1	1	1					0.5
GC 0080 Cereal grains	T5	5b, o, r, w	0	2 (corn 1)	0.5	0.5	0.5		(3 w, 1 bcor)	3	(5 wob)	0.5 (sr)
FS 0013 Cherries	5	10	10	10	3	1	1		1		10	1
~~VL 0465 Chervil~~	T10				1	1	1			10		
FC 0001 Citrus fruits	7	7	10	10	1	1	1	Mandarin 1	7		7	2
SO 0691 Cotton seed	1	1	5									
FI 0332 Custard apple	5				1	1	1					
FB 0266 Dewberries	10	10	12	10	1	1	1				10	0.5
MO 0105 Edible offal (mammalian)	T0.2		1									
PE 0112 Eggs	T0.2	0.5										
FI 0371 Elephant apple	5				1	1	1					
FI 0335 Feijoa	5				1	1	1					
VC 0045 Fruiting vegetables, Cucurbits	3	3c	10c, m	3	1	1	1		¥	4	(3 c m,p)	0.5€
~~HS 0783 Galangal, rhizomes~~	T5											
FI 0351 Granadilla	5				1	1	1					
FB 0269 Grapes	5	5	10	5	3	3	3	1	0.5		5	0.5
FT 0298 Grumichama [Brazilian cherry]	5				1	1	1					
FT 0336 Guava	5				1	1	1					
~~HH 0092 Herbs~~	T10											
FT 0300 Jaboticaba	5				1	1	1					
FI 0338 Jackfruit	5				1	1	1					
Jambu	5				1	1	1					
~~Kaffir lime leaves~~	T10											
FI 0341 Kiwifruit	10	10			1	10	1		10		1	0.1
VL 0053 Leafy vegetables	10	10	10cc, 12	Lettuce 10	1 ,lettuce 3	1, lettuce 3	1 (lettuce 3)		(lettuce 1)	10	10	1☼
~~Lemon grass~~	T10		mg									
~~DT 1111 Lemon verbena~~	T10											
FI 0343 Litchi	5				1	1	1					0.5
FI 0342 Longan	5				1	1	1					0.5
FI 0345 Mango	5				1	1	1					0.5
MM 0095 Meat [mammalian]	T0.2	0.2c, g										
ML 0106 Milks	T*0.05	*0.1	0.1									
~~Mizuna~~	T10		0.3									
FS 0245 Nectarine	10	10		10	3	1	1				10	1
VO 0442 Okra	10	10	10		1	1	1			4		0.5
FT 0305 Olives	10	10	10		1	1	1					
DM 0305 Olives, processed	1	1	10		1	1	1					

FI 0350 Papaya [pawpaw]	5		1		1	1	1					0.1
FI 0351 Passion fruit	5				1	1	1				5	0.1
FS 0247 Peach	10	10		10	3	3	3	1	10		10	1
FS 0014 Plums (including Prunes)	5	10	10	10	3	3	3		1		10	1
FP 0009 Pome fruits	5	5a, p	10	5	3	3	3	1	(1 a, 0.5 p)		5	1
VR 0589 Potato	0.2	0.2		0.2	0	0.1	1	0.1	0.2	0.2	0.2	
PO 0111 Poultry, Edible offal of	T5		0.2 (N)									
PM 0110 Poultry meat	T0.5	0.5										
FI 0358 Rambutan	5				1	1	1					0.1
FB 0272 Raspberries	10	10		10	1	1	1				10	0.5
~~VL 0496 Rucola (Rocket)~~	T10		12		1	1	1			10	10	
FI 0359 Sapodilla	5				1	1	1					
FI 0360 Sapote, Black	5				1	1	1					
FI 0361 Sapote, Green	5				1	1	1					
FI 0362 Sapote, Mammey	5				1	1	1					
FI 0363 Sapote, White [casimiroa]	5				1	1	1					
FB 0275 Strawberry	7	7		7	1	1	1		0.5		7	0.5
GS 0659 Sugar cane	T*0.05		10				1					
SO 0702 Sunflower seed	1											
VO 0447 Sweet corn (corn-on-the-cob)	1	1k	1		1	1	1			4	1	0.5
TN 0085 Tree nuts	1	1	5									
TN 0085 Tree nuts [whole in shell]	10		1									
~~HS 0794 Tumeric, root~~	T5											
Vegetables [unless specified]	5	5 ind	5 ind	(5¢)	1	1£	1 (3 cab)	1 bs, cab	§	4©	®	♣
CM 0654 Wheat bran, unprocessed	T20	20										
Fruit [unless specified]‡					1	1	1					

¢ pea, bean, carrot, tomato, peppers, celery
¥ Melon 3 ppm, pumpkin, squash 1 ppm , cucumber 0.5 ppm
§cabbage, eggplant, tomato, carrot 0.5 ppm; celery, pea 1 ppm
©vegetables 4 ppm except leafy vegetables 10 ppm, root and tuber vegetables 0.2 ppm
£sweet potato 0.1 ppm
®eggplant, tomato, peppers, beans, peas 5 ppm, carrots 2 ppm
€cucumber, bitter melon, luffa, wax gourd, pumpkin, vegetable pear etc but not melons such as watermelon, cantaloupe, melon etc
☼Chinese cabbage, mustard, Chinese mustard, Chinese kale, water spinach, spinach, lettuce, garland chrysanthemum, leaf beat etc
♣garlic, spring onion, Chinese leek, celery, cabbage, cauliflower, broccoli, Brussels sprouts 1 ppm; soybean, peanut, mungbean, small red bean etc, sweet potato, tomato, egg plant, sweet pepper, snap bean, snow pea, vegetable soybean, lablab, asparagus bean, kidney bean etc 0.5 ppm
‡(Drupe) mango, longan, litchi, loquat etc, (Small berries) grape, strawberries, carambola, persimmon, wax apple, guava etc, 0.5 ppm; (Large berries) banana, papaya, pineapple, kiwifruit, mangosteen, sweet sop, avocado, pitaya, passion fruit, durian, rambutan etc, 0.1 ppm