AUSTRALIAN PESTICIDES AND VETERINARY MEDICINES AUTHORITY

AUSTRALIA

CHEMICAL REVIEW PROGRAM

HUMAN HEALTH RISK ASSESSMENT

OF

CARBENDAZIM

Prepared by

Office of Chemical Safety and Environmental Health
Office of Health Protection

of the

Department of Health and Ageing

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PREFACE: This document is a human health risk assessment covering the public and occupational health issues associated with the use of carbendazim as a pesticide in Australia. The document is divided into two parts: Part I is an evaluation of the mammalian toxicology of carbendazim, , and is aimed at reviewing the existing public health standards to ensure that the continued use of carbendazim as a pesticide does not pose unacceptable risks to the general population. Part II of this document deals with the risks associated with the professional use of carbendazim and addresses the issue of residential/bystander exposure.

ABBREVIATIONS

Time

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

Weight

bw	Bodyweight
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)
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
BUN	Blood urea nitrogen
GGT	Gamma-glutamyl transpeptidase
Hb	Haemoglobin
Hct	Hematocrit
LDH	Lactate dehydrogenase
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
T3	triiodothyronine
T4	thyroxine
WBC	White blood cell/leucocyte

Chemistry

DMSO	Dimethyl sulfoxide
HPLC	High pressure liquid chromatography
TLC	Thin layer chromatography

Terminology

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
DFR	Dislodgeable Foliar Residue
GLP	Good Laboratory Practice
LOEL	Lowest Observed Effect Level
MOE	Margin of Exposure
MRL	Maximum Residue Limit or Level
NOEL	No Observed Effect Level
NOAEL	No Observed Adverse Effect Level
OHS	Occupational Health and Safety
OP	Organophosphorus pesticide
PPE	Personal Protective Equipment
REI	Re-entry interval
RHI	Re-handling interval
SD	Sprague Dawley (rats)
SPF	Specific pathogen free
WHP	Withholding period

Organisations & publications

ACP	Advisory Committee on Pesticides (UK)
APVMA	Australian Pesticides and Veterinary Medicines Authority
DoHA	Department of Health and Ageing
EC	European Commission
FAO	Food and Agriculture Organisation of the UN
FAISD	First Aid Instructions & Safety Directions
IARC	International Agency for Research on Cancer (UN)
IPCS	International Programme on Chemical Safety
JMPR	Joint Meeting on Pesticide Residues
NDPSC	National Drugs and Poisons Scheduling Committee
NHMRC	National Health and Medical Research Council
OCSEH	Office of Chemical Safety and Environmental Health
PHED	Pesticide Handlers Exposure Database
PMRA	Pesticide Management Regulatory Agency (Canada)
PSD	Pesticide Safety Directorate (UK)
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

EXECUTIVE SUMMARY

Carbendazim

Carbendazim is a broad-spectrum systemic fungicide with protective and curative action. Carbendazim products are used for the control of a wide range of fungal diseases such as mould, spot, mildew, scorch, rot and blight in a variety of crops. The target crops include fruit (eg. strawberries, pome fruit, stone fruit, citrus, mangoes, bananas and grapes), cucurbits, legumes, macadamia nuts, roses, ginger, sugar cane, pasture and turf. In addition, a few products are used as timber preservatives. Carbendazim products are not intended for home garden use.

At the commencement of this review, there were 21 registered carbendazim products. The label approvals of these products were suspended to add additional instructions to the labels pending the outcome of the review (as detailed on the APVMA website). Of the 21 products, 19 were suspension concentrates (17 contain 500 g/L and the other two containing 80-100 g/L carbendazim), one product was an emulsifiable concentrate (75 g/L) and one was a wettable powder (500 g/kg). As of December 2009, there were 18 products registered, however the original 21 products are included in this review.

Carbendazim was nominated for review under the Australian Pesticides and Veterinary Medicines Authority's (APVMA) Chemical Review Program because of concerns over its potential to cause birth defects and impair human fertility and the consequent risks to workers using carbendazim products. In reviewing this concern, the OCSEH examined all of the available data and concluded that carbendazim has the potential to cause birth defects and impair human fertility. These effects are considered to be an undue hazard to the safety of certain workers and therefore a warning statement should be required for products containing carbendazim. As these effects are observed above a threshold dose protected by the public and occupational health standards set for carbendazim in this review, there is no objection on public or occupational health grounds to the continued registration of all existing carbendazim products. However, a number of use patterns are no longer supported by the OCSEH, as set out in the recommendations of this review.

The current Australian ADI for carbendazim of 0.03 mg/kg/d based on a NOEL of 2.5 mg/kg bw/d from a 2-year dog study and incorporating a safety factor of 100, was established in 1979. Following a review of submitted and archived data, the current ADI for carbendazim remains appropriate. No acute reference dose (ARfD) for carbendazim had been previously established. An ARfD of 0.05 mg/kg/bw for carbendazim has been set in this review by applying a safety factor of 1000 to the LOEL of 50 mg/kg bw derived from a testicular toxicity study in rats. It was also recommended that the poison schedule of carbendazim be revised from S6 to S7 of the SUSDP, and that an exemption for inclusion in paints[1] at 0.5% or less was no longer appropriate. The NDPSC agreed with these recommendations at its 57th meeting, October 2009.

[1] Surface coatings (including paint but excluding antifouling paint) are not regulated by the APVMA. Schedule 3 of the *Agricultural and Veterinary Chemicals Code Regulations 1995*, "Substances declared not to be agricultural chemical products".

The OCSEH also recommended that all registered products containing carbendazim should bear the following warning statement: ***"Contains carbendazim which causes birth defects and (irreversible) male infertility in laboratory animals. Avoid contact with carbendazim".*** This review has also recommended a new health-based guideline value for carbendazim in drinking water of 0.09 mg/L.

No changes to the approval status of carbendazim have been proposed in this review. The review identified a number of additions and amendments to the existing Safety Directions for carbendazim products.

Operators mixing and loading SC 500 g/L and WP 500 g/kg carbendazim products should wear gloves because of the potential for slight skin irritation. In addition, during this process face shields or respirators should be worn to prevent accidental ingestion for the SC and WP formulations, respectively. Operators applying carbendazim are likely to be exposed mainly via the dermal and inhalational routes. Based on exposure modelling, even at the maximum anticipated daily work rates, spray operators applying carbendazim with airblast or groundboom equipment are not heavily exposed, and engineering controls are not required to protect them. Therefore, the OCSEH has no objections to the continuation of these uses when appropriate PPE is worn. It is likely that operators applying carbendazim to ornamental plants and turf by hand-held equipment will be significantly exposed to the chemical. Even with the application of PPE (gloves and overalls), this risk cannot be mitigated, Therefore, the OCSEH does not support these uses.

Carbendazim has a long half-life (up to 6 months) and therefore occupational re-entry exposure can occur for a significant length of time following application. This risk assessment demonstrates that re-entry exposure in grapes, stone fruits, custard apples, apples, pears, turf and roses is unacceptable, and these use patterns can no longer be supported. In addition, additional re-entry statements are required for pasture and red clover, and strawberries.

There is the potential for toxicologically significant dermal and oral exposure of the public (especially toddlers) to occur when using turf treated with carbendazim products. Therefore, application of carbendazim products on public places including parks, golf courses, bowling greens and other sporting fields, as well as on commercial turf, can no longer be supported.

CONSOLIDATED RECOMMENDATIONS TO THE APVMA FOR CARBENDAZIM

1. Approval Status

No change is recommended to the approval status of carbendazim.

2. Product Registration

There is no objection on public and occupational health grounds to the continued registration of several use patterns of existing carbendazim products. The use of carbendazim on ornamentals is no longer supported on occupational health and safety grounds, and the use on turf is no longer supported from both an occupational and public health perspective. From a re-entry perspective, use on grapes, stone fruits, custard apples, apples, pears, turf and roses is no longer supported.

3. Acceptable Daily Intake

The present review reaffirmed the current ADI for carbendazim of 0.03 mg/kg bw/d, based on a NOEL of 2.5 mg/kg bw/d from a 2-year dog study and applying a safety factor of 100. The NOEL is based on chronic hepatitis observed at the next highest dose (12.5 mg/kg bw/d) and is protective of developmental and testicular effects.

4. Acute Reference Dose

A new ARfD of 0.05 mg/kg/bw for carbendazim has been established by applying a safety factor of 1000 to the LOEL of 50 mg/kg bw derived from a study on testicular toxicity in rats.

5. Water Quality Guidelines

A new NHMRC health-based guideline value for carbendazim in drinking water of 0.09 mg/L is recommended.

6. Poisons Schedule

It was recommended that the poison schedule of carbendazim be revised from Schedule 6 to Schedule 7 of the SUSDP. The Committee decided to reschedule carbendazim to S7 at the October 2009 meeting. In addition, the exemption for paints, jointing compounds or sealants containing 0.5 per cent or less of carbendazim was no longer considered appropriate[2].

7. First Aid Instructions and Warning Statements

It is recommended that all registered products containing carbendazim should bear the following warning statement: ***"Contains carbendazim which causes birth defects and (irreversible) male infertility in laboratory animals. Avoid contact with carbendazim".***

The existing First Aid Instructions for carbendazim remain appropriate.

[2] Surface coatings (including paint but excluding antifouling paint) are not regulated by the APVMA.

8. Occupational Health and Safety Considerations

a. The OCSEH recommends that the APVMA should be satisfied that persons involved in preparing and applying carbendazim products, according to the revised label directions (details below), will not suffer from adverse effects.

b. Based on the likelihood of toxicologically unacceptable levels of dermal and oral exposure to the public, uses of carbendazim on parks, golf courses, bowling greens and other sport-playing fields, as well as on commercial turf, should cease.

c. The following uses of carbendazim are supported with minor changes to the Safety Directions which appear on the label (details below):

- Application to field crops by boom spray.
- Application to orchard crops by airblast.
- Application to plant materials by dipping.
- Application to timber by spraying and dipping.

d. The following uses of carbendazim are no longer supported, from an occupational health and safety perspective:
    - Application to ornamental plants and commercial turf by hand-held equipment.

e. The following uses of carbendazim are supported without changes to the current use pattern, based on re-entry risk assessment:
    - Cucurbits.
    - Chickpeas/faba beans/lentils.
    - Macadamia nuts.

Re-entry statement:

For cucurbits, chickpeas, faba beans and lentils, and macadamia nuts, the following re-entry statement is recommended on the product label:

"Do not allow entry into treated areas until the spray has dried, unless wearing cotton overalls buttoned to the neck and wrist (or equivalent clothing) and chemical resistant gloves. Clothing must be laundered after each day's use."

f. The following uses of carbendazim are supported with PPE specified for re-entry procedures:
    - Pasture/red clover.
    - Strawberries.

Re-entry statement:

For pasture and red clover, and strawberries, the following re-entry statements are recommended on the product label:

"Do not allow entry into treated areas until the spray has dried, unless wearing cotton overalls buttoned to the neck and wrist (or equivalent clothing) and chemical resistant gloves. Clothing must be laundered after each day's use."

"Do not allow entry into treated areas after the spray has dried, unless wearing chemical resistant gloves."

g. The following uses of carbendazim are no longer supported, based on unacceptable exposure during re-entry and extended re-entry intervals:
   - Grapes.
   - Stone fruits, custard apples, apples and pears.
   - Roses.
   - Turf.

9. Safety Directions

The following amended Safety Directions have been established. These will be included in the FAISD Handbook, and should be included on the product label.

Amended Entry

Carbendazim SC 500 g/L or less greater than 80 g/L	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
160 162 164	May irritate the eyes and skin
210 211	Avoid contact with eyes and skin
220 222 223	Do not inhale vapour or spray mist
279 280 281 282 290 294c 296	When opening the container and preparing spray or dip, wear elbow-length chemical resistant gloves and face shield.
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

Amended Entry

Carbendazim WP 500 g/kg or less	
	Safety Directions
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
160 162 163	May irritate the eyes and nose and throat
210 211	Avoid contact with eyes and skin
220 221 223	Do not inhale dust or spray mist
279 280 281 282 290 294c 301 302	When opening the container and preparing spray or dip, wear elbow-length chemical resistant gloves and a full facepiece respirator with dust cartridge or cannister.
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 364 366	After each days use, wash gloves, respirator and contaminated clothing

Amended Entry

Carbendazim SC 80 g/L or less with dodecylbenzene sulfonic acid 450 g/L or less and n-methyl-2-pyrrolidone 450 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 211	Will damage the eyes and skin
210 211	Avoid contact with the eyes and skin
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

Amended Entry

Carbendazim EC 75 g/L or less with zinc naphthenate 90 g/L or less and n-methyl-2-pyrrolidone 370 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 211	Will damage the eyes and skin
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

Amended Entry

Chlorothalonil SC 720 g/L or less with carbendazim 100 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if inhaled, absorbed by skin contact or swallowed
161 164	Will irritate the skin
207 162	Will damage the eyes
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
180	Repeated exposure may cause allergic disorders.
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

PART I: TOXICOLOGICAL & PUBLIC HEALTH ASSESSMENT OF CARBENDAZIM

TOXICOLOGY HAZARD PROFILE OF CARBENDAZIM

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of absorption	Almost complete absorption ~ (86%) in rats.
Distribution	In rats, the highest concentrations of benomyl/carbendazim after oral exposure were found in the eyes, blood, GIT, liver and kidney.
Potential for accumulation	No evidence of accumulation.
Rate and extent of excretion	Within 72 h of PO administration to rats, 86% excreted in urine, with the remainder in faeces (13%).
Metabolism	Main metabolite is 5-hydroxy carbendazim (5-HBC-S)
Toxicologically significant compounds (animals, plants and environment)	Carbendazim

Acute toxicity

Rat oral LD_{50} (mg/kg bw)	> 2000
Worst oral LD_{50} in other species	No data
Rat dermal LD_{50} (mg/kg bw)	> 2000
Worst dermal LD_{50} in other species	No data
Rat inhalation LC_{50} (mg/m^3)	> 4280
Worst inhalation LC_{50} in other species	No data
Skin irritation	Not irritant in rabbits
Eye irritation	Not irritant in rabbits
Skin sensitisation	Not sensitising in guinea pigs

Short-term toxicity

Target/critical effect	Testicular toxicity; premature release of immature germ cells, atrophy and decreased diameter of seminiferous tubules, abnormal growth of efferent ductules and increased frequency of micronuclei in spermatids
Lowest relevant oral NOEL (mg/kg bw/d)	No NOEL was established (LOEL 50 mg/kg bw) (single dose studies in rats)
Lowest relevant dermal NOEL (mg/kg bw/d)	10000 (3-wk study in rabbits)
Lowest relevant inhalation NOEC (mg/m^3)	No data

Genotoxicity	Genotoxic above a threshold dose. Carbendazim induces numerical chromosomal aberrations by disrupting mitotic spindle formation.

Long-term toxicity and carcinogenicity

Target/critical effect	Liver toxicity and perturbations in hepatic biochemistry. Testicular degeneration.
Lowest relevant NOEL (mg/kg bw/d)	2.5 (2-yr feeding study in dogs) for liver toxicity
Carcinogenicity	Induces hepatic cell proliferation leading to hepatocellular adenomas in mice. But not considered to be an appropriate model for the formation of hepatic tumours in humans. No evidence of interaction with DNA in genotoxicity testing.

Reproductive toxicity

Reproduction target/critical effect	Altered sperm production and morphology in rats
Lowest relevant reproductive NOEL (mg/kg bw/d)	No NOEL was established (LOEL 50 mg/kg bw/d). One generation reproduction study in rats and hamsters.

Developmental toxicity

Developmental target/critical effect	Reduced foetal bodyweight, micro-/anophthalmia and hydrocephalus in the absence of maternal toxicity in rats. Decreased implantation, increased resorption and decreased live litter size in the absence of maternal toxicity in rabbits.
Lowest relevant developmental NOEL (mg/kg bw/d)	10
Delayed neurotoxicity	No evidence of delayed neurotoxicity in hens
Immunotoxicity	No data
Dermal absorption	Approximately 4.5% over 4 h, human skin *in vivo*

Summary

	NOEL (mg/kg bw/d)	Study	Safety factor
ADI (0.03 mg/kg bw/d) [chronic hepatitis]	2.5	Chronic feeding study in dogs	100
ARfD (0.05 mg/kg/bw) [testicular toxicity]	50 (LOEL)	Testicular toxicity studies in rats	1000
NOEL for OHS assessment (5 mg/kg bw/d) [testicular toxicity]	50 (LOEL)	Reproduction study in rats	10

Health-based guideline value in drinking water	0.09 mg/L

SUMMARY OF MAIN FINDINGS

Carbendazim is a mitotic spindle poison which is capable of inducing numerical chromosomal aberrations following both mitotic and meiotic cell division. This aneugenic mechanism of action is considered to occur above a threshold dose and likely to be responsible for the testicular toxicity and teratogenicity of carbendazim and the parent compound benomyl. There is no evidence that carbendazim causes carcinogenicity.

Carbendazim induced teratogenic effects (head and eyes) were observed in rats following gavage dosing at 30 mg/kg bw/d (NOEL 10 mg/kg bw/d), however, exposure via the diet at greater than 700 mg/kg bw/d did not cause serious developmental/reproductive toxicity. A similar profile is seen for benomyl. The OCSEH considers that gavage dosing leads to a saturated metabolic pathway and high transient carbendazim concentrations crossing the placental barrier and affecting the developing foetus.

Testicular toxicity is considered to be another critical endpoint for carbendazim. In two studies in rats, a single dose of 50 mg/kg bw was sufficient to induce either an increase in the frequency of micronuclei in spermatids or premature release of germ cells two days post-exposure, atrophy of seminiferous tubules, decreased seminiferous tubule diameter and abnormal growth of efferent ductules. These effects persisted for at least 70 days post-exposure. No NOEL was established for testicular toxicity from the available studies.

HAZARD ASSESSMENT

Reasons for the Review

In 2004, OCSEH reviewed benomyl which is the parent compound of carbendazim. Benomyl is rapidly converted to carbendazim *in vitro* and *in vivo*. Because benomyl was found to cause foetal malformations and testicular toxicity in laboratory animals, OCSEH recommended the two structurally-related chemicals, carbendazim and thiophanate-methyl be included in the APVMA's Chemical Review Program. This consolidated report incorporates all toxicological evaluations on carbendazim completed by the Australian Department of Health and Ageing between 1983-2004 as well as some evaluations by the JMPR and IPCS. Unpublished studies from 1968 to 1990 and literature articles up to 2007 are also included in this is report.

The toxicological database on carbendazim is uneven in its coverage and quality. Apart from acute and genotoxicity studies, many of the studies date back to the early 1970s and do not conform to current test guidelines or standards of reporting. However there is extensive and more recent data on reproductive and developmental toxicity, the end points of greatest concern. Acute toxicity studies are only available for one 50% SC carbendazim formulation (Shincar 500 SC Fungicide). Taken as a whole, there is sufficient data to enable regulatory standards to be set for carbendazim. For comparative purposes and given their similar toxicological endpoints, benomyl studies evaluated in the OCSEH benomyl review (2004) will be cited throughout the discussion.

Toxicokinetics and Metabolism

In rats, benomyl is readily converted to carbendazim, with around 70% of the administered benomyl being converted to carbendazim within one hour (Sherman *et al* 1975). In rats,

carbendazim is readily absorbed after oral exposure. Once absorbed, carbendazim was extensively metabolised and excreted in the urine and faeces. Carbendazim's main metabolite is 5-hydroxy carbendazim (5-HBC-S). Hydroxylation can also occur in the 6- or 5-position to form MBC-5,6-diol. This hydroxide derivative can undergo further biotransformation to glutathione or glucuronide conjugates, while the secondary ring nitrogen can form the N-oxide derivatives. Approximately 98% of carbendazim and its metabolites are excreted from the body in three days. Tissue accumulation of carbendazim and its metabolites is low (less than 1%).

The metabolism, distribution and excretion of carbendazim are likely to have a major influence on its activity as a developmental toxin. In pregnant rats administered benomyl by gavage on d 7-16 of gestation, benomyl and carbendazim were found to cross the placental membrane. Levels of benomyl/carbendazim in maternal blood and embryonic tissue were greatest one h after dosing. The half-life of benomyl was approximately 45 min in maternal blood and shorter in embryos. Benomyl/carbendazim levels one hour post-dose decreased with increasing number of treatments, while the level of 5-HBC-S increased with repeated exposure (the half-life of 5-HBC-S was 2-3 h in maternal blood and 4-8 h in embryos), suggesting that repeated exposure leads to faster benomyl metabolism (Culik 1981b). Hepatic enzyme induction is the most probable cause.

When benomyl was administered to pregnant rats in the diet at a dose approximately 4-fold higher than given by gavage, benomyl/carbendazim was not detected in the embryos, and the peak level of benomyl/carbendazim in maternal blood was markedly lower than had been present after the onset of gavage administration (Culik 1981a). However, the peak concentrations of 5-HBC-S in maternal blood and embryos were approximately double those detected following gavage dosing. This indicates that benomyl administered via the diet is metabolised faster than by gavage.

Acute Toxicity

Carbendazim exhibited low acute oral, dermal and inhalational toxicity in rats. It was not a skin or eye irritant in rabbits. Carbendazim was not a skin sensitiser in guinea pigs. The product, Shincar 500 SC Fungicide (50% carbendazim) showed a similar toxicity profile.

Short-term Toxicity

A 13-week dietary study in rats reported a treatment related effect on liver weights at the highest dose tested of 67.5 mg/kg bw/d. The NOEL for this study was 22.5 mg/kg bw/d. Summaries of short-term and subchronic oral studies in rats and dogs evaluated by JMPR (2005) have been included in this evaluation but were not independently assessed by the OCSEH. JMPR (2005) reported reduced bodyweight gain, inhibition of spermatogenesis and atrophy of the testes in rats at doses of 815 mg/kg bw/d and above following short-term and subchronic exposure. In dogs, treatment related effects of increased plasma ALT and ALP levels, decreased plasma albumin levels, increased liver and thyroid weights and decreased heart weights were reported at doses of 25 mg/kg bw/d and above. The most sensitive NOEL reported for short-term and subchronic effects in dogs was 7.5 mg/kg bw/d, on the basis of minor changes in clinical chemistry and organ weights.

The critical endpoint for oral short-term toxicity is testicular toxicity observed in rats following a single gavage dose of 50 mg/kg bw, the lowest dose tested (see *testicular*

toxicity). The NOEL for this study is considered to be 5 mg/kg bw. As this NOEL is protective of effects in short-term and subchronic studies reported by JMPR (2005) independent assessment of these studies by OCSEH is not required.

Long-term and Carcinogenicity Studies

There have been conflicting findings with respect to the carcinogenicity of carbendazim. There was some evidence that carbendazim caused an increased incidence of hepatocellular carcinomas in one feeding study in mice at and above the lowest dose of 500 ppm (Wood 1982). However, other studies in mice did not support the carcinogenic effects of carbendazim (Bee *et al* 1976; Danaubauer *et al* 1982) at doses ≥ 500 ppm in the diet.

Liver tumours are known to develop spontaneously in many strains of mice, at relatively high incidence, without intentional exposure to chemicals (Haseman *et al* 1998). One study that employed the HOE NMRKf mouse strain, which is known to have a low background incidence of liver tumours (1-2%), did not provide evidence of oncogenicity when exposed to carbendazim at doses of up to 5000 ppm (Danaubauer *et al* 1982). Life-time studies have also been carried out in rats using both benomyl (Sherman *et al* 1969b) and carbendazim (Til *et al* 1976). Both studies were negative for oncogenicity at doses up to and including 2500 and 10000 ppm, respectively. Therefore the hepatic tumours observed in mice appear to be a species-related phenomenon, possibly consequent to enzyme induction.

Long-term feeding studies with carbendazim in dogs led to hepatotoxicity as shown by the elevation in ALP activity and increased liver weights at 5000 ppm in the diet (Reuzel *et al* 1976). This result was substantiated by similar findings in a benomyl chronic feeding study in beagle dogs (elevated liver enzymes and weight) at 2500 ppm in the diet (Sherman at al 1970). Changes in the weight of liver observed in these two studies were without corroborative gross morphological and histopathological findings suggesting that this is likely to be an adaptive response with little toxicological significance.

Long-term exposure to carbendazim in the di*et al*so revealed progressive testicular degeneration in rats and dogs. In a 2-year rat study (Sherman 1972), increased incidences of diffuse testicular atrophy and prostatitis were seen at 250 mg/kg bw/d. In a 2-year dog study (Reuzel *et al* 1976), increased incidences of prostatitis and atrophic tubules of the testes were observed at 50 mg/kg bw/d. These effects are discussed separately below.

Genotoxicity

There is an extensive scientific literature which demonstrates that carbendazim interacts with tubulin, disrupting microtubule assembly, preventing the formation of the cell division spindle and thus resulting in a failure of cell division. Concentrations of carbendazim which completely eliminate mitotic spindle formation in mammalian cells lead to the formation of "imperfect" mitotic spindles and thus to the mal-segregation of chromosomes, resulting in the production of aneuploid progeny cells including those with both reduced and increased chromosome numbers i.e. monosomic and trisomic.

With the exception of the chromosomes that determine sex (the *sex chromosomes*); a diploid nucleus contains two closely similar versions of each of the other chromosomes (the *autosomes*), one from the male parent (paternal chromosome) and one from the female parent (maternal chromosome). The two versions are called homologues, and in most cells they

maintain a completely separate existence as independent chromosomes. When each chromosome is duplicated by DNA replication during cell division, the twin copies of the fully replicated chromosome at first remain closely associated and are called sister chromatids. In an ordinary cell division the sister chromatids line up on the spindle during mitosis with their kinetochore fibres pointing toward opposite poles. The sister chromatids then separate from each other at anaphase to become individual chromosomes. In this manner each daughter cell formed by ordinary cell division inherits one copy of each paternal chromosome and one copy of each maternal chromosome. Occasionally, the meiotic process occurs abnormally and homologues fail to separate - a phenomenon known as non-disjunction. In this case some of the haploid cells that are produced lack a chromosome, while others have more than one copy. This can give rise to micronuclei which are small, extranuclear bodies that arise from acentric chromosome fragments or from whole chromosomes that are excluded from the nucleus during mitotic cellular division (i.e. unable to attach to the spindle at mitosis). Micronucleus induction is an indirect indicator of mutagenicity. It is unclear however, whether micronucleus formation has a specific role in carcinogenesis.

In mammalian cells, the cell division spindle is a critical structural component responsible for the accurate segregation and distribution of chromosomes during both mitotic and meiotic cell division. Both mitosis and meiosis involve the polymerisation of tubulin, assembly of microtubules and the formation of a cell division spindle. The cellular target of carbendazim interactions are essentially the same for both mitosis and meiosis. However, there is currently available no evidence to prove conclusively that the concentrations of carbendazim which modify mitosis and meiosis are identical.

Carbendazim has been adequately tested in a range of assays for genotoxicity. The genotoxic effects were seen in tests for the induction of micronuclei or aneuploidy *in vivo* after single dose of 50 mg/kg bw (Matsuo *et al* 1999). Similar to its parent compound benomyl, carbendazim did not cause gene mutations, structural chromosomal damage or interact directly with DNA in mammalian studies employing either somatic or germ cells. Carbendazim does however, cause numerical chromosome aberrations (aneuploidy and/or polyploidy) in *vitro* in human lymphocytes (Marshall *et al* 1996), mouse oocytes (Can & Albertini 1997) and *in vivo* in rat sperm (De Stopplaar 1999) and Syrian hamster oocytes (Costa *et al* 2001). In cultured human lymphocytes, carbendazim/benomyl induced aneuploidy at similar concentrations ≥ 0.2/0.25 µg/mL respectively (Elhajouji *et al* 1995, 1997; Bentley *et al* 2000). This is considered the threshold for aneugenic activity for carbendazim/benomyl. Carbendazim and benomyl fed to ICR mice lead to mitotic arrest and abnormal mitoses, such as bridge formation, lagging chromatin and tripolar anaphases when dosed twice at 1000 mg/kg bw (Seiler 1976). Additionally, benomyl treatment led to the formation of micronuclei in bone marrow of mice at doses ≥ 1000 mg/kg bw (Seiler 1976; Sasaki 1990) and induced hyperploidy in mouse oocytes *in vivo* (Mailhes & Aardema 1992; Sarrif 1994b). These observations are consistent with the proposed mode of action of carbendazim/benomyl as mitotic spindle poisons

Colcemid, a known potent mitotic spindle poison, disrupts spindle apparatus in mice resulting in chromatin loss due to chromosomal breakage as well as loss of entire chromosomes. In cultured human lymphocytes, colcemid and carbendazim treatment resulted in similar C-metaphase morphology. Although colcemid was 200-fold more effective in inducing C-metaphases, polyploidies and micronuclei, carbendazim induced longer-lasting spindle damage that resulted in higher frequency of polyploidies in subsequent mitoses in comparison

to colcemid (Banduhn & Obe 1985). Benomyl has also been shown to exhibit the same threshold concentration as carbendazim (3.2-4.3 μM) for chromosomal non-disjunction and aneuploidy (Bentley *et al* 2000).

There is sufficient evidence from mechanistic and mouse micronucleus studies to indicate that carbendazim and benomyl can reach and interact with the microtubules of the spindle apparatus of germ and somatic cells, inducing numerical chromosomal aberrations. This is illustrated by the observed testicular toxicity (discussed in the following section) and the induction of micronuclei in bone marrow cells. In 1999, the European Commission's group of Specialised Experts in the fields of carcinogenicity, mutagenicity and nephrotoxicity concluded that there was enough evidence to classify benomyl and carbendazim in mutagenicity category 2 [Muta. Cat 2; R46] based on the observed aneugenic effects.

Testicular Toxicity

A number of studies have shown that both benomyl and carbendazim treatment leads to similar toxic effects in the testes (Table 1). The LOEL for testicular effects following a single dose of carbendazim was 50 mg/kg bw and no NOEL has been determined. The reproductive toxicity of benomyl/carbendazim involves the reduction of testicular and epididymal weights together with reduced epididymal sperm counts and reduced fertility (Hess *et al* 1991; Nakai *et al* 1992; Gray *et al* 1990). Histological examinations indicate that there is seminiferous tubular atrophy, early sloughing (premature release) of the germ cells and occlusion of the efferent ductules following carbendazim exposure (Hess *et al* 1991; Gray *et al* 1990). One of the initial responses in the testis after benomyl/carbendazim exposure is the reduction of spermatogenesis seen histologically as the sloughing of germ cells in division, as early as 3-6 h (mitotic) and 12 h (meiotic) following exposure (Hess & Nakai 2000). Apart from sloughing of germ cells, alterations in the nucleus formation of spermatids have also been noted (Hess *et al* 1991; Hess & Nakai 2000; Gray *et al* 1990). Due to disruption of microtubule formation, benomyl/carbendazim prevents pachytene spermatocytes from completing the second meiotic division, leading to enlarged spermatids (30% greater size than normal spermatids) in the tubules (Hess *et al* 1991; Nakai 1997). These enlarged spermatids or aneuploid spermatogenic precursor cells contain near-diploid amounts of DNA and can develop to be mature spermatozoa, despite their genetic defect (Hess & Nakai 2000). Thus benomyl/carbendazim exposure has the potential to cause chromosomal abnormalities in an embryo or developing foetus.

Table 1: Summary of findings on testicular toxicity for both benomyl and carbendazim

Study Type & Species	Study Author	LOEL (mg/kg bw/d)	Study Duration (d)/Dose Route	Effects
Benomyl*				
Reproduction, rat	Sherman & Krauss (1966)	3400	Single dose/gavage	Sloughing of germinal epithelium evident up to 5 d. Depletion of germ cells with slight – marked oligospermia at 5-14 d post-treatment. Effects were not reversible in some rats.
	Hess *et al* (1991)	100	Single dose/gavage	Germinal epithelial sloughing and tubular atrophy, not reversible after 70 d.
	Dashiell (1978)	200	10/gavage	Degeneration of the germinal epithelium. ↓ spermatogenesis, reversing in some animals. ↓ fertility, reversing after 59 d recovery.
	Carter & Laskey (1982)	200	10/gavage	↓ Epididymis weight.
	Barnes *et al* (1983)	20	70/diet	↓ sperm count, reversible after 70 d recovery.
	Linder *et al* (1988)	45	62/gavage	↓ testicular weight and sperm production. No effect on fertility.
	Kavlock *et al* (1982)	31.2	GD 7 to LD 15/gavage (dams)	↓ testicular, seminal vesicle & prostate weights in progeny at 100 d old.
	Mebus (1991)	168 (P1)	70 (P1)/diet	Oligospermia, fertility unchanged.
		234 (F1)	105 (F1)/diet	Testicular atrophy and degeneration, oligospermia, fertility unchanged.
Chronic, mouse	Wiechman (1982)	500	2-yr/diet	Degeneration of seminiferous tubules, aspermatogenesis, atrophy, distended acini.
Chronic, dog	Sherman *et al* (1970a)	12.5	2-yr/diet	Oligospermia, spermatogenesis, marked testicular degeneration.
Carbendazim				
Effect on male reproductive tract, rat	Nakai *et al* (1992)	50	Single dose/gavage	Premature release of immature germ cells 2 days post exposure, atrophy of seminiferous tubules, decreased seminiferous tubule diameter and abnormal growth of efferent ductules
Induction on micronucleus, rat	Matsuo *et al* (1999)	50	Single dose/gavage	Increased frequencies of micronuclei in spermatid
Reproduction, rat	Gray *et al* (1990)	50	83/gavage	Mild testicular atrophy, ↓ sperm counts, ↓ altered sperm morphology
Reproduction, hamster	Gray *et al* (1990)	400	gavage	16-17% lower sperm count
Subchronic, rat	Scholz & Weigand (1972)	1000	30/diet	Inhibition of spermatogenesis
	Scholz & Schultes (1973)	780	90/diet	Small testes and atrophy of seminiferous tubuli
Chronic, rat	Sherman (1972)	250	2-yr/diet	Diffuse testicular atrophy
Chronic, dog	Reuzel *et al* (1976)	125	2-yr/diet	Atrophic tubules of the testes

*From OCSEH Benomyl Review (2004)

In rats treated with a single gavage dose of either benomyl or carbendazim at 100 mg/kg bw and above, there was a rapidly induced sloughing of the germinal epithelium, followed by occlusion of the efferent ductules and seminiferous tubular atrophy (Hess *et al* 1991; Nakai *et al* 1992). These effects were only partially reversible over a 70-180 d interval after treatment. At 50 mg/kg bw, carbendazim caused premature release of rat immature germ cells 2 days post exposure, atrophy of seminiferous tubules, decreased seminiferous tubule diameter and abnormal growth of efferent ductules (Nakai *et al* 1992). Carbendazim also increased the frequencies of micronuclei in rat spermatids after a single gavage dose of 50 mg/kg bw, the lowest dose tested (Matsuo *et al* 1999).

It is clear from the studies in rats and dogs that benomyl/carbendazim exposure leads to some degree of testicular toxicity, depending on the dose and duration of exposure. Mechanistic studies in laboratory animals demonstrate that carbendazim interferes with microtubule polymerisation and has rapid direct effects on meiotic spermatocytes and latent effects on spermatids. There is no evidence that these effects would not occur in humans. The epidemiological study in humans performed by Gooch (1978, 1979) found no effect on human fertility, but this study was poorly designed. The induction of testicular toxicity is an important toxicological endpoint that may have implications for male workers manufacturing or using benomyl/carbendazim technical and/or their products.

There is strong evidence to indicate that carbendazim, rather than benomyl, is responsible for the testicular toxicity of benomyl. This is evidenced from experiments in which equimolar concentrations of benomyl and carbendazim were administered to rats, either intraperitoneally or by direct injection into the testis (Lim & Miller, 1997). Whereas no significant testicular damage was observed both 1 and 2 hours after benomyl administration by the interperitoneal route, carbendazim administration resulted in sloughing of the seminiferous epithelium after 1 hour, which increased in severity at the 2-hour time point. Furthermore, intratesticular treatment with benomyl caused little testicular damage after 1 hour whereas an equimolar amount of carbendazim produced severe disruption of the seminiferous epithelium. Testicular levels of carbendazim and benomyl were measured at various times after both routes of administration. Using an area under the curve (AUC) to express testicular concentration levels over two 2 hours, a good linear correlation was observed between sloughing and AUC for carbendazim. A similar correlation was also observed for benomyl, although it was less potent. This is further reflected with the observation that the concentration which reduced microtubular assembly in the testis by 50% was 5 µM (1µg/ml) for carbendazim and 75 µM (15µg/ml) for benomyl.

Reproductive and Developmental Toxicity

Most gavage administration of benomyl or carbendazim induced both testicular changes that resulted in reduced fertility and teratogenic effects with a predominance of head and eye malformations, whereas most dietary studies have shown only minor signs of reproductive toxicity at high doses and no effects on development in the absence of maternal and/or paternal toxicity.

In a reproduction study by Gray *et al* (1990) where rats (and hamsters) were dosed with carbendazim by gavage at doses of 50, 100, 200 or 400 mg/kg bw/d (and 0 and 400 mg/kg bw/d, respectively), no NOEL was established based on effects on sperm production and morphology at the lowest dose. In rats, doses of 200 and 400 mg/kg bw/d resulted in severe

testicular atrophy and very low sperm counts and subsequently reduced numbers of pregnant females and lower foetal viability. At the higher dose there were no viable litters and at 200 mg/kg bw/d one in three litters, and at 100 mg/kg bw/d two in seven litters, had malformations (hydrocephaly), with most pups dying at 200 mg/kg bw/d. Sperm quality and morphology were altered at all doses. In the same study, hamsters treated with 400 mg/kg bw/d were much less affected, although males had 17% lower sperm count in the testis and 16% lower in the cauda epididymis, but there was no effect on fertility.

In a study designed to assess potential maternal effects of carbendazim during early pregnancy in rats to distinguish maternal from embryotoxic effects and to differentiate between early pregnancy failure and late embryonic loss, doses up to 1000 mg/kg bw/d were administered by gavage through days 1-8 of pregnancy (Cummings *et al* 1990). The results showed that doses of carbendazim which are teratogenic when administered in late pregnancy do not produce pregnancy failure. Carter *et al* (1987) performed a serial breeding technique in rats, where males were exposed to 400 mg/kg bw/d daily by gavage over 10 days and then bred weekly with different untreated females for 32 weeks. Male fertility was reduced during the first mating (10/24 failed to result in pregnancy) and by the fifth week 16/24 failed to produce pregnancy and only 4 of these males recovered fertility, the remainder remaining infertile. Histologically, this latter group exhibited severe seminiferous tubular atrophy. Those males that recovered fertility had various contents of atrophic tubules 245 days after treatment. Another reproductive study in rats was evaluated, but was considered not suitable for regulatory purposes.

In developmental studies where rats were dosed via gavage, foetal malformations were observed at doses between 30–90 mg/kg bw/d, and in all cases with no signs of maternal toxicity. Foetuses exhibited various skeletal malformations, which included anophthalmia, hydrocephaly (distended lateral ventricles), and fused vertebrae sternum and ribs. Similar developmental effects in rats were also observed with benomyl at doses between 10-125 mg/kg bw/d. In the study by Alvarez (1987), rats were gavaged with carbendazim at doses of 0, 5, 10, 20 or 90 mg/kg bw/d which resulted in hydrocephalus and anophthalmia (absence of the eye) at ≥ 90 mg/kg bw/d. In the study by Hohmann & Peh (1987b), pregnant rats were gavaged with carbendazim at doses 0, 10 or 30 mg/kg bw/d, there were 17 cases of hydrocephaly at 30 mg/kg bw/d [NOEL = 10, LOEL = 30 mg/kg bw/d].

Although single-dose developmental studies in rats are not available, given that benomyl is rapidly converted to carbendazim *in vivo*, it is of interest to discuss the developmental effects caused by benomyl after a single oral dose. Vergieva (1998) reported exophthalmia, microphthalmia, hydrocephaly and related malformations in rat foetuses whose dams were gavaged at 62.5 mg/kg bw/d from gestation days (GD) 6-15. The NOEL for repeat-dose administration was 15.6 mg/kg bw/d. They observed that single gavage doses of benomyl can cause more severe developmental effects than repeated doses. Dilation of the lateral brain ventricles occurred at 15.6 mg/kg bw/d in response to a single gavage dose on GD 13. At the next highest dose of 62.5 mg/kg bw/d, the incidence of brain and cranio-facial deformities was approximately 3-fold higher following a single dose on GD 13 than after daily administration of the same dose from GD 6 - 15. They also reported higher incidences of embryo lethality in response to single doses of benomyl administered on GD 9 or 11 compared with GD 7 or 13. Conversely, developmental effects were more severe when dams were treated with benomyl on GD 13 than on GD 11 or earlier. This implies that the sensitivity of the rat foetus to differing toxicological end-points changes through the various stages of embryo- and organogenesis.

The enzyme-inducing effect of benomyl observed by Guengerich (1981) may also explain the apparently enhanced potency of single doses of benomyl, compared with repeated equivalent doses. In a standard repeat-dose protocol, the dam would receive 7 consecutive doses before GD 13, when the foetus is maximally sensitive to developmental effects. The onset of enzyme induction during the first half of gestation could therefore increase maternal metabolism and/or excretion of benomyl, thereby reducing the concentration at (or duration over) which foetal exposure occurs during the critical period of organogenesis.

In contrast to the teratogenic effects occurring in response to gavage administration, the older dietary study (Culik *et al* 1970) showed no evidence for embryo- or foetotoxicity or teratogenicity when pregnant rats were given feed containing carbendazim at concentrations of up to 747 mg/kg bw/d. The influence of the mode of oral administration on teratogenic effects was also noted in benomyl studies. Major malformations in rats were not seen in dietary studies conducted with benomyl at up to 500 mg/kg bw/d (Kavlock *et al* 1982), yet in gavage studies, gross foetal malformations (hydrocephaly, micro-/anophthalmia, malformed scapulae) were noted at ≥ 10 mg/kg bw/d (Staples *et al* 1980; Alvarez 1985).

Culik (1981a & 1981b) has shown that despite a 4-fold difference between the doses, the concentration of benomyl/carbendazim in maternal rat blood following repeated dietary administration of benomyl at 600 mg/kg bw/d is similar to levels following repeated gavage dosing at 125 mg/kg bw/d. This suggests that the presence of food in the GIT slows the absorption of benomyl, and may explain why exposure to benomyl at comparatively high doses in the diet produces only minor effects such as delayed ossification. When benomyl was administered to pregnant rats in the diet at a dose approximately 4-fold higher than given by gavage, benomyl/carbendazim was not detected in the embryos, and the peak level of benomyl/carbendazim in maternal blood was markedly lower than had been present after the onset of gavage administration (Culik, 1981a). However, the peak concentrations of 5-HBC in maternal blood and embryos were approximately double those detected following gavage dosing. It is possible that the teratogenic effects resulting from benomyl gavage exposure may be due to a saturated metabolism pathway, leading to high transient benomyl/carbendazim concentrations crossing the placental barrier and affecting the developing foetus. The presence of relatively high levels of 5-HBC in maternal blood and embryos after both gavage administration (which causes teratogenicity in rats) and dietary administration (which does not), suggests that this metabolite does not play a significant role in developmental toxicity.

In rabbits given carbendazim via gavage, embryotoxicity was observed at doses ≥ 20 mg/kg bw/d and teratogenicity occurred at 125 mg/kg bw/d (Christian *et al* 1985). However, unlike in rats, compound-related malformations consisted of malformed cervical vertebrae and interrelated malformation of the ribs and proximate thoracic vertebrae, but not anophthalmia or hydrocephaly.

Normal embryonic development is characterised by rapid and coordinated cell replication. Thus, it follows that mitotic interference is a potential mechanism underlying chemically-induced developmental effects. Carbendazim binds to and induces conformational changes within brain tubulin (Russel *et al*, 1992). Its effects on foetal development may be related to its ability to bind to tubulin and thus prevent tubulin polymerisation, which interrupts spindle formation during cell division. Perturbation in the mitotic spindle may result in cell cycle arrest, numerical chromosomal aberrations (aneuploidy) as well as alterations in cell division

rate and/or cell death. This mechanism of action would be expected to exhibit greatest adverse effects on rapidly dividing cells such as occurs during foetal development.

Carbendazim may mediate developmental toxicity through an additional mechanism by disrupting the outgrowth of neurites, a process that is dependent on formation of microtubule bundles. Neurites are elongated, membrane-enclosed protrusions of cytoplasm that are formed by neurons during the development of the nervous system within the embryo. The neurites grow towards other regions of the nervous system or other structures (such as glands or muscle) on which the neurons will form synapses. Most neurites eventually become functional axons, and therefore neurite formation is a crucial process in establishing the physical connections via which nerve impulses are transmitted during early brain development.

McLean *et al* (1998) have shown that benomyl interferes with the differentiation of human and mouse cancer cells *in vitro* by disrupting the outgrowth of neurites. Hence, the hypothesis that benomyl (or carbendazim) may mediate teratogenic effects by disturbing neurite outgrowth is plausible, even though it is unclear whether the neuroblastoma cells utilised by McLean *et al* are more or less sensitive to this effect than the developing nerve cells and tissues normally present in embryos. Little information is available on the inter-relationship between CNS malformations (or malformations of other tissues) and deficits in the formation of neurites *in vivo*. In particular, it is not known to what extent neurite outgrowth must be inhibited before foetal malformation will occur, and there are insufficient data to establish the threshold oral or dermal dose of carbendazim required to cause biologically significant inhibition of neurite outgrowth in embryos.

Neurotoxicity

The neurotoxicity database for carbendazim comprises of one fairly old acute study in hens, performed at single doses of up to 5000 mg/kg bw (Goldenthal 1978). Ataxia and leg weakness occurred at 5000 mg/kg bw. These clinical signs were reversible upon cessation of treatment and microscopic examination indicated no axonal degeneration or demyelination in carbendazim-treated animals. The various repeat-dose studies and chronic toxicity studies with in rodents and dogs have not yielded any evidence that benomyl/carbendazim mediates neurotoxicity, nor has any such evidence been reported in humans in the available scientific literature. Therefore, while a modern neurotoxicity study in rats would increase the assurance that benomyl/carbendazim is not neurotoxic; at least at probable levels of human exposure; this review has not raised any significant concerns in this regard.

Dietary Exposure Considerations

The 1999-2000 Australian National Residue Survey, conducted under the auspices of the Department of Agriculture, Fisheries and Forestry Australia (AFFA[3]), monitored benomyl residues in Macadamia nuts, apples and pears (the residue definition for benomyl included carbendazim and thiophanate-methyl). While no residues were found in Macadamia nuts (Limit of reporting = 0.1 mg/kg), 145/317 analyses detected residues in apples and pears. However, none of the samples contained residues at or greater than the MRL of 5 mg/kg. [4]

The 1992 and 1994 Australian Market Basket Surveys (AMBS) did not monitor residues of thiophanate-methyl, carbendazim or benomyl. Results from the dietary exposure assessment of benomyl/carbendazim emanating from the 19th Australian Total Dietary Survey (2001) are shown in Table 2. The mean dietary intake of benomyl/carbendazim represents less or equal to 0.59% of the ADI of 0.03 mg/kg bw/d and less than or equal to 0.36% of the proposed ARfD of 0.05 mg/kg bw.

Table 2: Human dietary exposure to benomyl and carbendazim

Group	Mean Dietary Intake (ng/kg bw/d)	% of ADI (0.03 mg/kg bw/d)	% of ARfD (0.05 mg/kg bw)
Adult males	45.03	0.15	0.09
Adult females	47.80	0.15	0.095
Boys 12 yr	101.96	0.34	0.2
Girls 12 yr	73.41	0.24	0.15
Toddlers 2 yr	179.63	0.59	0.36
Infants 9 months	34.74	0.11	0.07

The MRLs for carbendazim for some food commodities are quite high. For example, the MRL in citrus and stone fruits is 10 mg/kg. Using a worst case exposure scenario of a 60-kg pregnant woman or a 70-kg man ingesting 0.5 kg of citrus fruits or stone fruits in a single day, she/he may receive a potential dose of up to 0.08/0.07 mg carbendazim/kg bodyweight which would exceed the ADI and ARfD for carbendazim[5]. This is of concern since foetal malformations and testicular effects can potentially occurs after limited exposure to carbendazim.

The MRLs established for carbendazim were historically based on information which elicited no serious health concerns. The data presented in this review demonstrate that carbendazim can cause foetal malformations and testicular toxicity in rats and there is no evidence to indicate that these effects will not occur in humans. In view of this, the current MRLs for carbendazim will need to be re-examined in relation to their continued support as based on the toxicological profile of carbendazim.

[3] Now the Australian Government Department of Agriculture Fisheries and Forestry

[4] In 2008-2009 the NRS results note that samples were collected from apples (471), pears(136), macadamia nut (204), almonds (64), onion(123), sorghum (360), soybean (6), sunflower (19), triticale (6), wheat (2254) , wheat products (321), durum wheat (35), semolina (8)and durum wheat bran (8). Of these none (0/4015) detected carbendazim at levels equal to or above half the relevant Australian Standard (MRL) which for apples and pears was 2.5 mg/kg and for all other crops was 0.1 mg/kg or less.

[5] The APVMA suspended carbendazim product labels and issued new instructions prohibiting the use of carbendazim on these crops in Jan 2010

A dietary risk assessment, performed by the APVMA and FSANZ, will be reported separately.

DOSE LEVELS RELEVANT FOR PUBLIC HEALTH RISK ASSESSMENT

To identify the lowest NOELs for the establishment of an ADI and ARfD, a summary of the NOELs determined in those oral dosing studies considered suitable for regulatory purposes are shown in Table 3.

Table 3: Studies on carbendazim relevant for the establishment of an ADI and an ARfD

Species (study type)	NOEL (mg/kg bw)	LOEL (mg/kg bw)	Toxicological Endpoint	Reference
Single dose studies				
Rat (gavage)	-	50	Testicular toxicity	Nakai *et al* (1992)
Rat (gavage)	-	50	Testicular toxicity	Matsuo (1999)
Subchronic studies				
Rat (90 days, diet)	163	780	Small testes and atrophy of seminiferous tubuli	Scholz & Schultes (1973)
Rat (90 days, diet)	22.5	67.5	Increased liver weight	Hunter (1973)
Dog (90 days, diet	7.5	50	Increased liver and thyroid weight	Til (1972)
Chronic Studies				
Swiss mice (80 weeks, diet)	45	150	Increased liver weight	Beems (1976)
CD-1 mice (2 years, diet)	75	225	Increased liver weight, centrilobular hypertrophy, necrosis, and swelling	Wood (1982)
NMRKf mice (2 years, diet)	45	750	Increased liver weight, centrilobular hypertrophy, single cell necrosis,	Donaubauer (1982)
Rat (2 years, diet)	125	250	Diffuse testicular atrophy	Sherman (1972)
Rat (2 years, diet)	15	100	Increased liver weight	Til (1976a)
Dog (2 years, diet)	7.5	125	Atrophic tubules of the testes, prostatitis	Reuzel *et al* (1976)
Dog (2 years, diet)	2.5	12.5	Hepatic cirrhosis, swollen, vacuolated hepatic cells, and mild chronic hepatitis	Sherman (1972)
Developmental studies				
Rat (gavage)	30 (dams) 10 (foetuses)	60 (dams) 30 (foetuses)	Dam: Reduced bodyweight gain. Foetus: Reduced weight, increased incidence of malformation	Hofmann & Peh (1987a)*
Rat (gavage)	20 (dams) 10 (foetuses)	90 (dams) 20 (foetuses)	Dam: Reduced bodyweight gain. Foetus: Reduced weight, increased incidence of malformation	Alvarez (1987)*
Rat (diet)	747 (for both dams and foetuses)	-	Dam: Reduced bodyweight gain. Foetus: Reduced weight, increased incidence of malformation	Culik *et al* (1970)*
Rabbit (gavage)	20 (dams) 10 (foetuses)	125 (dams) 20 (foetuses)	Dam: Reduced bodyweight gain, increased abortion. Foetus: Reduced implantation and live litter size, increased resorption.	Christian *et al* (1985)*

* Reproduced from JMPR (2005) report. Original data not independently assessed by OCSEH.

PUBLIC EXPOSURE ASSESSMENT

Residues in Food and Drinking Water

In Australia, carbendazim is registered for use in a wide variety of situations for the control of fungal diseases in a variety of crops. The target crops include fruit (eg. strawberries, pome fruit, stone fruit, citrus, mangoes, bananas and grapes), cucurbits, legumes, macadamia nuts, roses, ginger, sugar cane, pasture and turf. In addition, a few products are used as timber preservatives. Carbendazim is also used in some house paints at 0.5%[6].

Chronic Dietary Intake

This issue has been discussed in the discussion section under the heading "Dietary Exposure Considerations".

Residues in Drinking Water

Based on its current pattern of use, exposure of the general population to carbendazim residues in drinking water is considered toxicologically insignificant.

Re-entry Exposure of the General Public

The general public may be exposed to carbendazim following re-entry onto treated turf areas (eg. golf courses, bowling greens, parks and sporting fields). In addition, the public may be exposed to treated turf laid in the home garden. At the time of this review, there were 17 SC products registered for professional use on turf. All 17 products are applied at a maximal rate of 0.3 g carbendazim/m^2.

Re-entry exposure to the general public is considered in Part III, Section 5 of this review.

HUMAN RISK ASSESSMENT

Dietary Risk Assessment

The dietary risk assessment for carbendazim will be performed by the APVMA and FSANZ.

Re-entry Risk Assessment

The risk assessment for re-entry of the general public onto treated turf is covered in the OHS review of carbendazim (See Part III).

[6] Surface coatings (including paint but excluding antifouling paint) are not regulated by the APVMA.

CONSIDERATION OF PUBLIC HEALTH STANDARDS

Approval Status

There is no objection on toxicological grounds to the ongoing approval of the carbendazim active constituents listed in Appendix I.

Impurity Limits

An integral part of the safety assessment of an active constituent is a consideration of the chemical composition of the material. Technical-grade active constituents will contain measurable levels of impurities, which can arise during manufacture and/or from subsequent degradation during storage. The chemical identity of these impurities is generally well characterised. The impurities present in the technical-grade material are usually of no particular concern since health standards are established on the basis of toxicology studies conducted using the mixture. However, for those impurities which have high acute toxicity, genotoxicity or teratogenic potential, concentration limits need to be set, so that the toxicological profile of the technical-grade active constituent does not appreciably alter in the event of slight changes in the proportions of the impurities.

From the declarations of composition from the four manufacturers, the active constituent carbendazim contains no impurities of toxicological concern.

Acceptable Daily Intake (ADI)

The ADI for humans is the level of intake of a chemical that can be ingested daily over an entire lifetime without appreciable risk to health. It is calculated by dividing the overall NOEL for the most sensitive toxicological endpoint from a suitable study (typically an animal study) by an appropriate safety factor. The magnitude of the safety factor is selected to account for uncertainties in extrapolation of animal data to humans, intraspecies variation, the completeness of the toxicological database and the nature of the potential toxicologically-significant effects.

The current Australian ADI for carbendazim is 0.03 mg/kg bw/d, based on a NOEL of 2.5 mg/kg bw/d established in a 2-year dog study using a safety factor of 100. Long-term exposure to carbendazim in the diet revealed progressive testicular degeneration in rats and dogs. In a 2-year rat study (Sherman 1972), a NOEL of 25 mg/kg bw/d was established based on an increased incidence of diffuse testicular atrophy and prostatitis at 250 mg/kg bw/d. In a 2-year dog study (Reuzel *et al* 1976), a NOEL of 7.5 mg/kg bw/d was established based on an increased incidence of prostatitis at 50 mg/kg bw/d (3/4 versus 1/4). In addition, 1/4 male dogs at that dose also had interstitial mononuclear inflammatory cell infiltrates and atrophic tubules of the testes. In another 2-year dog study (Sherman 1972), a lower NOEL of 2.5 mg/kg bw/d was established, based on liver toxicity at 12.5 mg/kg bw/d. This NOEL is considered the appropriate NOEL for the establishment of an ADI for carbendazim. Furthermore, this NOEL would also provide protection for testicular toxicity seen in the other dog study (Reuzel *et al* 1976). An application of a 100-fold safety factor to this NOEL results in an ADI of 0.03 mg/kg bw/d. Thus, following a review of all submitted and archived data, a

more suitable study was not identified. Therefore, the existing ADI for carbendazim remains appropriate.

Acute Reference Dose (ARfD)

The ARfD is the estimate of the amount of a substance in food or drinking water, expressed on a milligram per kilogram bodyweight basis, which can be ingested over a short period of time, usually one meal or one day, without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation.

An Australian ARfD has not been established for carbendazim; however, there are two toxicity endpoints which are relevant to establishing an ARfD for carbendazim, namely developmental and testicular toxicity. In two rat gavage developmental toxicity studies (Hofmann & Peh 1987a; Alvarez 1987), increased incidence of malformations affecting the head, spine and ribs were seen at doses of 20 mg/kg bw/d and higher. These occurred in the absence of maternal toxicity and the NOEL for these effects was 10 mg/kg bw/d. Applying a 100-fold safety factor would result in an ARfD of 0.1 mg/kg bw.

However, testicular toxicity was observed in rats following a single gavage dose of 50 mg/kg bw, the lowest dose tested (Nakai *et al* 1992). These include premature release of immature germ cells 2 days post-exposure, atrophy of seminiferous tubules, decreased seminiferous tubule diameter and abnormal growth of efferent ductules. This is further supported by another rat study in which increased frequencies of micronuclei were observed in spermatids after a single gavage dose of 50 mg/kg bw, the lowest dose tested (Matsuo *et al* 1999). Since testicular toxicity can potentially arise following a single exposure, it is considered to be an appropriate toxicological endpoint to establish an ARfD. A 1000-fold safety factor, incorporating 10-fold each for intra and interspecies variation and an additional factor of 10, to account for the use of a LOEL. On this basis, an ARfD of 0.05 mg/kg bw is established for carbendazim. This ARfD would also provide adequate protection for developmental toxicity, which can potentially arise following a single exposure.

Water Quality Guidelines

The Australian Drinking Water Guidelines (ADWG) is a joint publication of the National Health and Medical Research Council (NHMRC) and the former Agricultural and Resource Management Council of Australia and New Zealand[7]. The ADGW are not legally enforceable but rather provide a standard for water authorities and State health authorities to ensure the quality and safety of Australia's drinking water.

The *guideline value* (mg/L) is analogous to an MRL in food and is generally based on the analytical limit of determination. If a pesticide is detected at or above this value, then the source should be identified and action taken to prevent further contamination. The *health-based guideline value* (also expressed as mg/L) is intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide. The health values are derived so as to limit intake *from water alone* to approximately 10% of the ADI, on the assumption that (based on current knowledge) there

[7] Now the Natural Resource Management Ministerial Council (NRMMC)

will be no significant risk to health for an adult weighing 70 kg having a daily water consumption of 2 L over a lifetime.

Given that the NOEL for long-term exposure to carbendazim is 2.5 mg/kg bw/d, the health-based guideline value may be calculated as follows:

$$0.\ 09\ \text{mg/L} = \frac{2.5\ \text{mg/kg bodyweight per day} \times 70\ \text{kg} \times 0.1}{2\ \text{L/day} \times 100}$$

where:

- 2.5 mg/kg bw/d is a NOEL based on a 2-year dog study with carbendazim.
- 70 kg is taken as the average weight of an adult.
- 0.1 is a proportionality factor based on the assumption that 10% of the ADI will arise from the consumption of drinking water.
- 2 L/day is the estimated maximum amount of water consumed by an adult.
- 100 is the safety factor applied to the NOEL derived from animal studies. This safety factor incorporates a factor of 10 for interspecies extrapolation and 10 for intraspecies variation.

Hence, a health-based guideline value of 0.09 mg/L for carbendazim is recommended.

Poisons Scheduling

At the commencement of the review, carbendazim was included in Schedule 6 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP), except when in paint at concentrations of 0.5% or less. The NDPSC originally placed carbendazim in S6 in 1983. The following points should be noted in relation to the poison schedule of carbendazim:

- Carbendazim has very low acute oral, dermal and inhalational toxicity in rats. It is not a skin or eye irritant in rabbits. Carbendazim is not a skin sensitiser in guinea pigs.

- Carbendazim is used as an agricultural fungicide in a wide variety of crops, to which it is applied by either ground or aerial spray. Carbendazim products are used for the control of a wide range of fungal diseases such as mould, spot, mildew, scorch, rot and blight in a variety of crops. The target crops include fruit (pome fruit, stone fruit, citrus, strawberries and grapes), legumes, cucurbits, macadamia nuts, ornamentals (roses), pasture and turf. In addition, post-harvest uses include apples, bananas, citrus, mangoes, pears, rockmelons and stone fruit. Pre-planting uses are for ginger seed and sugar cane. Carbendazim products are also for use as timber preservatives and some house paint may contain carbendazim[8].

- Carbendazim has low acute oral, dermal and inhalation toxicity in rats. It causes no skin or eye irritation in rabbits. Carbendazim is not a skin sensitiser in guinea pigs.

- In repeat-dose studies with carbendazim by oral administration, the principal target organs were the liver and male reproductive system. Carbendazim caused liver injury and testicular degeneration and atrophy in rats and dogs. Dogs were especially sensitive to reproductive toxicity, which occurred at doses down to 50 mg/kg bw/d. The ADI for

[8] Surface coatings (including paint but excluding antifouling paint) are not regulated by the APVMA.

carbendazim (0.03 mg/kg bw/d) is based on a NOEL of 2.5 mg/kg bw/d for liver injury in dogs. Carbendazim was not carcinogenic in mice or rats.

- Adverse effects on male reproduction were confirmed in a number of studies in rats. Spermatogenesis was reduced at doses ranging down to 50 mg/kg bw/d, and irreversible testicular effects were noted at 100 mg/kg bw and above.

- When administered by gavage to rats, carbendazim is a developmental toxin capable of inducing severe malformations in the absence of maternal toxicity. Abnormalities including anophthalmia, hydrocephaly and skeletal deformities have consistently occurred in studies in rats. The NOEL for these effects is 10 mg/kg bw/d, with the LOEL by repeat-dose gavage administration being 30 mg/kg bw/d. The ARfD for carbendazim (0.05 mg/kg bw) is based on a LOEL of 50 mg/kg bw for testicular toxicity in rats. Although single-dosed developmental studies in rats are not available, it should be noted that the parent compound benomyl, which is rapidly converted to carbendazim *in vivo,* induces hydrocephaly in rat foetuses in response to a single gavage dose of 15.6 mg/kg bw on gestation day 13.

- Carbendazim's effects on the male reproductive system and foetal development are probably related to its inhibition of tubulin association, which interrupts spindle formation during cell division. Normal embryonic development is characterised by rapid and coordinated cell replication. Thus, it follows that mitotic interference in cells is a potential mechanism underlying chemically-induced developmental effects. Disruption of microtubule formation in spermatocytes is the likely mechanism causing effects on spermatogenesis. Tubulin inhibition is an aneugenic mechanism as it leads to the formation of "imperfect" mitotic spindles and thus to the mal-segregation of chromosomes, resulting in the production of aneuploid progeny cells including those with both reduced and increased chromosome numbers i.e. monosomic and trisomic. There is substantial evidence that carbendazim induces aneuploidy and polyploidy at doses similar to those that induced teratogenicity in rats. This aneugenic mechanism of action is considered to occur above a threshold concentration. There is no evidence that carbendazim can cause structural chromosomal damage or gene mutations.

- Carbendazim may present a hazard to the fertility of male agricultural workers. Consequently, the OCESH will be recommending a warning statement to be included on the product label.

- Carbendazim may present a developmental hazard to the foetuses of pregnant female agricultural workers. Consequently, the OCSEH will be recommending a warning statement to be included on the product label.

- Of concern also is the low amount required to be ingested to reach the new ARfD of 0.05 mg/kg bw. This equates to 3.5 mg of carbendazim for a 70 kg worker. Accidental ingestion during the use of agricultural products is considered high risk during the mixing/loading stage so the OCSEH has recommended face shields during this process.

- To summarise, at comparatively moderate doses carbendazim is a reproductive toxin in males, and is a teratogen that can potentially cause severe and irreversible malformations in the foetus without concomitant maternal toxicity. Reproductive toxicity has been demonstrated following administration of single doses of carbendazim. These effects

probably arise from interference with cellular division and differentiation, which has been demonstrated in cultured cells at physiologically relevant concentrations of carbendazim. The OCSEH does not object to the approval of carbendazim technical or registration of products containing the chemical, provided that the consequent risks are managed appropriately. The toxicity profile of carbendazim, in particular its developmental toxicity, appeared incompatible with its Schedule 6 status and it was recommended that carbendazim be placed in Schedule 7 of the SUSDP.

At its 57th meeting, on 20-21 October 2009, the NDPSC agreed that carbendazim be included in Schedule 7 of the SUSDP, and that the exemption for paints, jointing compounds and sealants containing 0.5% or less carbendazim was no longer appropriate (NDPSC 2009[9]).

First Aid Instructions

Existing first aid instructions for carbendazim as they appear in the First Aid Instruction and Safety Directions (FAISDs) Handbook are as follows:

Code	***First Aid Instruction***
a	If poisoning occurs, contact a doctor or Poisons Information Centre. *Phone Australia* 131126

This existing statement remains appropriate and no changes are recommended.

Warning Statements and General Safety Precautions

In view of the irreversible developmental effects observed in the absence of any maternotoxicity following oral administration, and potentially occurring after a single exposure, Warning Statement 26 is appropriate, i.e. 'Contains carbendazim which causes birth defects in laboratory animals. Women of child bearing age should avoid contact with carbendazim'.

In view of the irreversible testicular effects, the APVMA may consider including a warning statement for male infertility.

To incorporate both effects the following phrase is recommended for inclusion on the product label in Section 8:

"Contains carbendazim which causes birth defects and (irreversible) male infertility in laboratory animals. Avoid contact with carbendazim".

Safety Directions

At the commencement of this review, there were 21 registered carbendazim products. The label approvals of these products were suspended to add additional instructions to the labels pending the outcome of the review (as detailed on the APVMA website). Of the 21 products, 19 were suspension concentrate (17 containing 500 g/L and the other two containing 80-100

[9] NDPSC (2009) Record of Reasons of Meeting 57- October 2009. National Drugs and Poisons Schedule Committee.

g/L carbendazim), one product was an emulsifiable concentrate (75 g/L) and one was a wettable powder (500 g/kg).

Except for the product Shincar 500 SC Fungicide (low acute, dermal and inhalation toxicity, not a skin or eye irritant; not a skin sensitiser), no product toxicity data was available, so the assessment of toxicity was made on the basis of the available toxicity of the constituents and their respective concentrations in the products (Appendix III).

The safety directions for Australian carbendazim products are discussed in part III (Occupational Health and Safety Assessment).

CONCLUSIONS AND RECOMMENDATIONS

1. Approval Status

No change is recommended to the approval status of carbendazim.

2. Product Registration

Based on unacceptable risk to workers or the public, a number of use pattens/crops for carbendazim products are no longer supported by the OCSEH. For the remaining uses and crops there is no objection to the continued registration of existing carbendazim products, with revised label directions.

3. Acceptable Daily Intake

The present review reaffirmed the current ADI for carbendazim of 0.03 mg/kg bw/d, based on a NOEL of 2.5 mg/kg bw/d for liver toxicity in a 2-year dog study.

4. Acute Reference Dose

The present review established an ARfD for carbendazim of 0.05 mg/kg bw, based on a NOEL of 50 mg/kg bw for testicular toxicity in a rat study.

5. Water Quality Guidelines

A new NHMRC health-based guideline value for carbendazim in drinking water of 0.09 mg/L is recommended.

6. Poisons Schedule

It was recommended that carbendazim be placed in Schedule 7 of the SUSDP on the grounds that the chemical is both a developmental toxicant and fertility toxicant in laboratory animals in the absence of maternal toxicity and that the mechanism of toxicity may be relevant to humans. In addition, it was recommended that the exemption for paints containing 0.5% or less of carbendazim could no longer be supported, based on the small amount required to reach the new ARfD. At its 57th meeting, on 20-21 October 2009, the NDPSC agreed that carbendazim be included in Schedule 7 of the SUSDP, and that the exemption for paints was no longer appropriate[10].

7. First Aid Instructions and Safety Directions

This existing First Aid Instructions statement remains appropriate and no changes are recommended.

The following amended Safety Directions are recommended, which will be included in the FAISD Handbook, and which should be included on the product label.

Amended Entry

Carbendazim SC 500 g/L or less greater than 80 g/L	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
160 162 164	May irritate the eyes and skin
210 211	Avoid contact with eyes and skin
220 222 223	Do not inhale vapour or spray mist
279 280 281 282 290 294c 296	When opening the container and preparing spray or dip, wear elbow-length chemical resistant gloves and face shield.
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

Amended Entry

Carbendazim WP 500 g/kg or less	
	Safety Directions
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
160 162 163	May irritate the eyes and nose and throat
210 211	Avoid contact with eyes and skin
220 221 223	Do not inhale dust or spray mist
279 280 281 282 290 294c 301 302	When opening the container and preparing spray or dip, wear elbow-length chemical resistant gloves and a full facepiece respirator with dust cartridge or cannister.
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 364 366	After each days use, wash gloves, respirator and contaminated clothing

[10] Surface coatings (including paint but excluding antifouling paint) are not regulated by the APVMA.

Amended Entry

Carbendazim SC 80 g/L or less with dodecylbenzene sulfonic acid 450 g/L or less and n-methyl-2-pyrrolidone 450 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 211	Will damage the eyes and skin
210 211	Avoid contact with the eyes and skin
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

Amended Entry

Carbendazim EC 75 g/L or less with zinc naphthenate 90 g/L or less and n-methyl-2-pyrrolidone 370 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 211	Will damage the eyes and skin
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

Amended Entry

Chlorothalonil SC 720 g/L or less with carbendazim 100 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if inhaled, absorbed by skin contact or swallowed
161 164	Will irritate the skin
207 162	Will damage the eyes
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
180	Repeated exposure may cause allergic disorders.
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

8. Warning Statement

The following Warning Statement should be incorporated on the label:

"Contains carbendazim which causes birth defects and (irreversible) male infertility in laboratory animals. Avoid contact with carbendazim".

9. Occupational Health and Safety Considerations

a.. The OCSEH recommends that the APVMA should be satisfied that persons involved in preparing and applying carbendazim products, according to the revised label directions (details below), will not suffer from adverse effects.

b. Based on the likelihood of toxicologically unacceptable levels of dermal and oral exposure to the public, uses of carbendazim on parks, golf courses, bowling greens and other sport-playing fields, as well as on commercial turf, should cease.

c. The following uses of carbendazim are supported with minor changes to the Safety Directions which appear on the label (details below):

- Application to field crops by boom spray.
- Application to orchard crops by airblast.
- Application to plant materials by dipping.
- Application to timber by spraying and dipping.

d. The following uses of carbendazim are no longer supported, from an occupational health and safety perspective:
    - Application to ornamental plants and commercial turf by hand-held equipment.

e. The following uses of carbendazim are supported without changes to the current use pattern, based on re-entry risk assessment:
    - Cucurbits.
    - Chickpeas/faba beans/lentils.
    - Macadamia nuts.

Re-entry statement:

For cucurbits, chickpeas, faba beans and lentils, and macadamia nuts, the following re-entry statement is recommended on the product label:

"Do not allow entry into treated areas until the spray has dried, unless wearing cotton overalls buttoned to the neck and wrist (or equivalent clothing) and chemical resistant gloves. Clothing must be laundered after each day's use."

f. The following uses of carbendazim are supported with PPE specified for re-entry procedures:
    - Pasture/red clover.
    - Strawberries.

Re-entry statement:

For pasture and red clover, and strawberries, the following re-entry statements are recommended on the product label:

"Do not allow entry into treated areas until the spray has dried, unless wearing cotton overalls buttoned to the neck and wrist (or equivalent clothing) and chemical resistant gloves. Clothing must be laundered after each day's use."

And

"Do not allow entry into treated areas after the spray has dried, unless wearing chemical resistant gloves."

g. The following uses of carbendazim are no longer supported, based on unacceptable exposure during re-entry and extended re-entry intervals:
    - Grapes.
    - Stone fruits, custard apples, apples and pears.
    - Roses.
    - Turf.

MAIN TOXICOLOGY REPORT

1. INTRODUCTION

Carbendazim is the primary metabolite of thiophanate-methyl and benomyl. The latter was reviewed by OCSEH in 2004. Carbendazim is a broad-spectrum systemic fungicide with protective and curative action. It is absorbed through the roots and green tissues, with translocation acropetally and acts by inhibiting development of the fungal germ tubes, the formation of appressoria and the growth of mycelia. Carbendazim products are used for the control of a wide range of fungal diseases such as mould, spot, mildew, scorch, rot and blight in a variety of crops. The target crops include fruit (eg. pome, stone, citrus, strawberries, bananas, mangoes, grapes), cucurbits, legumes, ginger, sugar cane, macadamia nuts, roses, pasture and turf. In addition, a few products are used as timber preservatives.

Carbendazim was nominated for review based on concerns over their potential to cause impairment of reproduction and development and due to a number of critical OHS issues (risks arising from exposure during handling and application; re-entry exposure risks; and determination of appropriate personal protective clothing requirements).

Initially carbendazim was reviewed together with thiophanate-methyl (as this compound is rapidly converted to carbendazim in plants and the environment). However the thiophanate-methyl review has been finalised separately as it was found that thiophanate-methyl appears to undergo only very limited metabolic conversion to carbendazim in mammals and does not induce a similar impairment of reproduction and development.

1.1 Public Health Considerations of Carbendazim in Australia

The history of public health considerations in Australia for benomyl/carbendazim was extensively discussed in the OCSEH benomyl review (2004).

ADI

At the commencement of this review the Australian ADI for carbendazim, was 0.03 mg/kg/d based on a NOEL of 2.5 mg/kg bw/d from a 2-year dog study. This was established in 1979.

ARfD

At the commencement of this review no acute reference dose (ARfD) had been established for carbendazim.

Poisons Scheduling

In 1983, the NDPSC considered that carbendazim was a mutagenic compound and agreed that it be removed from Appendix B and placed in Schedule 6. In 1990, the NDPSC agreed to an exemption cut-off for paints containing 0.5 per cent or less of carbendazim from Schedule 6. In 2008, the Committee broadened this exemption to include jointing compounds and sealants.

Drinking Water Guidelines

When a pesticide is registered for use in water or in water catchment areas, the Joint Committee of the Agricultural and Resource Management Council of Australia and New Zealand and the NHMRC set guideline values and health-based guideline values for the pesticide 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. No guideline value in drinking water has been established for carbendazim.

Health-based guideline values are intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse 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-based guideline value for carbendazim is 0.1 mg/L (NHMRC, 2004[11]).

1.2 International Toxicology Assessments

US EPA

In October 2005 the US EPA published a Reregistration Eligibility Decision (RED) for thiophanate-methyl and its main metabolite carbendazim. It was determined that the acute and chronic dietary risk from residues of carbendazim in food and water was considered to be low. An acute RfD of 0.1 mg/kg bw/d was set for women of childbearing age, based on a NOAEL of 10 mg/kg bw/d for foetal malformations in a developmental toxicity study in rats and using a 100-fold uncertainty factor (analogous to a safety factor). For the general population, including children, an ARfD of 0.17 mg/kg bw/d was established based on a LOAEL of 50 mg/kg bw in a study of toxicity to the male reproductive system in rats (Nakai *et al* 1992) and an uncertainty factor of 300.

Dietary intake estimates indicated that none of the population exceeded the acute RfD. A chronic reference dose (analogous to an ADI) was set at 0.025 mg/kg bw/d, based on a NOAEL of 2.5 mg/kg bw/d for liver toxicity in a 2-year dog study and using a 100-fold uncertainty factor. The highest chronic dietary intake estimates were 26% of the ADI for the highest exposed population subgroup, children (1-6 years). Risk of exposure to residues in drinking water was not of concern.

Carbendazim was classified as a possible human carcinogen by the US EPA based on hepatocellular tumours in female CD-1 mice (Wood *et al* 1982).

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR)

[11] National Health and Medical Research Council (2004), Australian Drinking Water Guidelines Canberra, Australia; ISBN online: 1864961244 or http://www.nhmrc.gov.au/_files_nhmrc/file/publications/synopses/adwg_11_06.pdf).

The JMPR evaluated the toxicology of carbendazim in 1973, 1976, 1977, 1978, 1983, 1985, 1995 and 2005. In 1995, the JMPR established an ADI of 0.03 mg/kg bw/d based on a NOEL of 2.5 mg/kg bw/d in a 2-year dog study and a safety factor of 100. In their 2005 consideration of carbendazim, the JMPR evaluated developmental toxicity studies that clearly demonstrated that carbendazim is a developmental toxicant and teratogen in rats. An ARfD of 0.1 mg/kg bw for women of childbearing age based on a NOEL of 10 mg/kg bw/d in rat and rabbit developmental toxicity studies and a safety factor of 100. For the general population, including children, an ARfD of 0.5 mg/kg bw was established based on a NOEL of 50 mg/kg bw in a study of toxicity to the male reproductive system in rats (Nakai *et al* 1992) and a safety factor of 100.

UK

The UK Advisory Committee on Pesticides (ACP) considered the review of carbendazim (together with benomyl and thiophanate-methyl) in March 1992 and concluded that one of the main concerns was the aneugenic activity of carbendazim and compounds that are metabolised to carbendazim (eg benomyl). The committee considered that the [then] available published data were not sufficient to establish a NOEL for aneuploidy. In order to set an ADI for these compounds, the sponsor was requested to provide a modern mouse micronucleus study, and an *in vitro* study using cultured mammalian cells to establish whether a NOEL could be established for aneuploidy. A developmental toxicity study in rabbits, a dermal penetration study and epidemiological data were also requested (UK PSD, 1992). Professional and home garden approvals were allowed to continue until the new data were received. The documents submitted by DuPont Australia do not explain the apparent revision of the previous recommendation to suspend approval of benomyl products for amateur use.

In March 1993, following the publicised allegations of a link between exposure to benomyl and anophthalmia, the ACP considered the study of Hoogenboom *et al* (1991), and concluded that the study was designed to test the mechanisms of action of benomyl at high doses "and under extreme conditions". The results were judged to be consistent with those considered previously by the committee, which had indicated a clear safety level for birth defects. The ACP therefore reiterated its previous assessment that there was no cause for concern over the continued use of benomyl under the conditions applying in the UK (UK MAFF, 1993), a position which remained unchanged following review of *in vitro* studies tendered by lawyers acting for the plaintiff in US litigation against DuPont (UK PSD, 1996).

The UK review of methyl benzimidazole carbamate fungicides concluded that threshold concentrations of 1000 and 500 ng/mL could be regarded as *in vitro* NOELs for aneuploidy caused by benomyl and carbendazim, respectively, that the NOEL for developmental effects in rats was 30 mg/kg bw/d, and that no developmental toxicity had occurred in rabbits at the highest dose tested (180 mg/kg bw/d). It was considered that the eye and brain malformations in benomyl-exposed rat foetuses could be related to the mechanism of benomyl-induced aneuploidy, i.e. binding of the chemical to tubulin. Although the *in vitro* data could not be used directly for regulatory purposes, the ACP considered that it was possible to set an ADI and acceptable operator exposure level (AOEL) values for benomyl and carbendazim, based on the lowest NOAEL in *in vivo* toxicology studies, in conjunction with a suitable uncertainty factor. However, it was recommended that no further action should be taken under the UK national review, and that the ADI and AOEL should be set as part of the EU review of

benomyl and carbendazim (UK ACP, 1997). Pending the outcome of the EC review, approval for all uses of benomyl and carbendazim should be allowed to continue.

Canadian Pest Management Regulatory Agency (PMRA)

In September 2005, the Canadian PMRA conducted a preliminary risk assessment of thiophanate-methyl and carbendazim. An ARfD of 0.05 mg/kg bw was set for males, based on a LOAEL of 50 mg/kg bw in a study of toxicity to the male reproductive system in rats (Nakai *et al* 1992) and an safety factor of 1000. An acute ARfD of 0.01 mg/kg bw was established for females of child-bearing age, based on NOAEL of 10 mg/kg bw/d in a developmental toxicity study in rats (Hofmann & Peh 1987b) and using a 1000-fold safety factor. The NOAEL was based on increased foetal malformations. An ADI of 0.009 mg/kg bw/d was set, based on a NOAEL of 9 mg/kg bw/d in a 2-year dog dietary study (reference not stated) and a safety factor of 1000. An additional 10-fold safety factor was applied for both ARfD and ADI because of foetal sensitivity and severity of effects.

European Commission

In February 2005, the European Commission conducted a review of toxicology for thiophanate-methyl and carbendazim. An ARfD and an ADI of 0.02 mg/kg bw/d were established, based on NOAEL of 10 mg/kg bw/d in a developmental toxicity study in rats (Hofmann & Peh 1987b) and using a 500-fold safety factor.

Carbendazim has been listed by the European Commission on a priority list of chemicals that are believed to affect hormone function. According to this listing, carbendazim is ED category 2[12]:

> "Based on potential for endocrine disruption. *In vitro* data indicating potential for endocrine disruption in intact organisms. Also includes effects *in vivo* that may, or may not, be ED-mediated. May include structural analyses and metabolic considerations".

It is also classified as a high production volume chemical (HPV) and is not considered persistent (EC, 2000[13]).

International Agency for Research on Cancer (*IARC)*

Carbendazim has not been evaluated by IARC.

IPCS

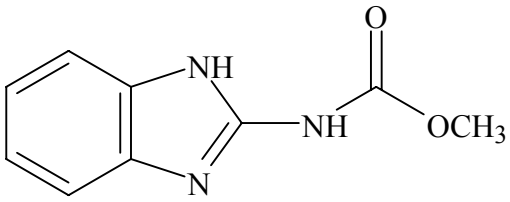
[12] Category 2 compounds (such as carbendazim) have been given a lower priority for further investigation than Category 1 substances. Category 1 substances have been assessed by the EU as having clear evidence for endocrine disruption in an intact organism .

[13] EC (2000) Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption: preparation of a candidate list of substances as a basis for priority setting. Final Report, 21 June 2000. Annex 10: List of 564 substances with their selection criteria.
<http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm#priority_list>
EC (2007) Study on enhancing the Endocrine Disruptor priority list with a focus on low production volume chemicals ENV.D.4/ETU/2005/0028r. Appendix L Updated ranked priority list.

In 1995, the International Programme on Chemical Safety (IPCS) of the WHO evaluated the toxicology of carbendazim. An ADI of 0.03 mg/kg bw/d was established for carbendazim based on a NOEL of 2.5 mg/kg bw/d in a 2-year dog study. An AfRD was not set.

1.3 Chemistry –Active Constituent - Carbendazim

Common name:	Carbendazim (ISO Approved)
Chemical name:	Methyl-[benzimidazol-2-yl] carbamate (IUPAC)
CAS Registry Number:	10605-21-7
Empirical Formula:	$C_{19}H_{18}N_4O_3$
Molecular Weight:	191
Chemical structure:	



Chemical class: Benzimidazole

Structural analogues: Benomyl, methyl thiophanate

Chemical and physical properties

Colour:	White crystalline solid
Odour:	Odourless
Physical state:	Crystalline solid
Melting point:	302-307^{o}C
Density (20^{0}C):	1.45 g/cm^3
Partition coefficient: (log K_{ow})	1.49
Vapour pressure:	0.15 mPa at 25^{o}C
Solubility in water:	8 mg/L at pH 7 and 20^{0}C
Solubility in organic solvents:	
Hexane	0.5 mg/L
Benzene	36 mg/L
Ethanol	300 mg/L
Acetone	300 mg/L
Chloroform	200 mg/L

Technical active - Declaration of Composition and Batch Analysis

Declarations of composition for technical grade carbendazim are shown in Appendix II. At the commencement of the review there were eight approval holders for carbendazim.

Impurities of Toxicological Concern

The active carbendazim contains no impurities of toxicological concern, with the APVMA's minimum compositional standard specifying a minimum carbendazim content of 980 g/L.

1.4 Products

At the commencement of the review there were 21 registered carbendazim products. See part III for more details.

2. METABOLISM AND TOXICOKINETICS

Since no studies were submitted, the following studies are reproduced from the IPCS toxicological evaluation for carbendazim published in 1993 (IPCS Environmental Health Criteria 149). The studies by Culik (1981a, b) and Guengerich (1981) are reproduced from the OCSEH benomyl review (2004). A general metabolic pathway for carbendazim is shown in Figure 1. The metabolism and toxicokinetics of the parent compound benomyl was discussed in the OCSEH benomyl review (2004).

Sherman H, Culik R, & Jackson RA (1975) Reproduction, Teratogenic, and Mutagenic Studies with Benomyl. Toxicol. Appl.. Pharmacol. 32: 305-315

[2-^{14}C]-Benomyl (0.322 Ci/mg, unspecified source and purity) in corn oil (unspecified source) was administered to one male ChR-CD rat by intra-gastric intubation at a dose level of approximately 900 mg/kg bw. Blood was collected and analysed for radioactivity. One hour after dosing, the blood contained less than 15% of administered radioactivity in the form of the parent compound and 68% [2-^{14}C]-carbendazim. This result indicates that benomyl is readily converted to carbendazim *in vivo*.

***Christ, O. & Kellner, H.M. (1973) Animal tests with carbendazim. Unpublished report from Hoechst AG, Frankfurt, Germany*.**

In this study, ^{14}C-Carbendazim administered by gavage to rats at 2 mg/kg bw/d for 10 consecutive days was cleared from the blood rapidly, and 59% of the radiolabel was excreted in the urine and 36% in the faeces. Elimination was biphasic, with a rapid rate during the first three days and a slower phase thereafter. Residues in the liver represented 0.3% of the administered dose seven days after the last administration and 0.08% after 14 days. The levels in blood and organs other than the liver (kidney, fat, muscle, and gonads) did not exceed 0.03% of the administered dose after seven days.

Krechniak, J. & Klosowska, B. (1986) The fate of ^{14}C-carbendazim in the rat. Xenobiotica, 16: 809-815.

Male albino rats were given a single oral dose of 12 mg/kg bw ^{14}C-carbendazim as a solution in diethyl glycol-ethanol. Urinary excretion of ^{14}C-carbendazim and two of its metabolites indicated that about 85% had been absorbed. Rats were also given a single dose of 12 mg/kg bw ^{14}C-carbendazim as a solution in diethyl glycol ethanol by intravenous injection. The highest concentrations of radiolabel were found in kidney and the lowest in blood; elimination followed the kinetics of a two-compartment model. By 12 hours, only small quantities of radiolabel were present in blood, liver, and kidney.

Monson K.D. (1990) Metabolism of [phenyl(U)-^{14}C]carbendazim in rats. Unpublished report from E.I. Du Pont de Nemours and Co., Inc. Wilmington, Delaware, USA.

Three groups of five rats of each sex were given [phenyl(U)-^{14}C]-carbendazim by gavage: one group received a single dose of 50 mg/kg bw; the second received a single dose of 50 mg/kg bw after 14 days of pre-treatment with 50 mg/kg bw/d unlabelled carbendazim; and the third received a single dose of 1000 mg/kg bw labelled carbendazim. In all groups, > 98% of the recovered radiolabel had been excreted in the urine or faeces by the time of sacrifice 72 hours after treatment. Urinary excretion accounted for 62-66% of the dose in males and 54-62% of

the dose in females at the low dose with or without pre-treatment. In animals at the high dose, this pathway accounted for 41% of the dose. The total recovery from faeces represented about 24% for males and 33-38% for females at the low dose with or without pre-treatment and > 60% for males and females at the high dose. Unchanged carbendazim represented 10-15% of the administered dose in the faeces of rats at the high dose. There were no apparent differences between male and female rats with respect to the extent of absorption or the extent or rate of elimination of ^{14}C-carbendazim equivalents within each dose group. The label remaining in tissues represented was less than 1% of the administered dose. The metabolite 5-HBC-S (see Figure 1) was identified as the main metabolite (43% of the dose), except in females at the high dose or receiving pre-treatment (5.5-10%); in all groups of females, 5,6-HOBC-N-oxide was the predominant metabolite (10-19%). 5,6-DHBC-S and 5,6-DHBC-G were identified as minor metabolites.

Dorn, E. & Keller, H.M. (1980) Carbendazim (60% wettable powder) absorption via the skin in rats. Unpublished report from Hoechst AG, Frankfurt, Germany.

Percutaneous absorption of carbendazim is negligible. In rats that were given 0.6 mg over 10% of the body surface, only about 0.2% of a radio labelled dose was excreted in urine and faeces within 24 h. When 60 mg per rat were applied under similar conditions, only 0.03% was excreted.

Dorn E., Schmidt E., Kellner H.M. & Leist K.H. (1983) HOE 017411-14-C (carbendazim-^{14}C) metabolic fate in rats and mice, a comparison. Unpublished report from Hoechst AG, Frankfurt, Germany.

NMRI mice and Wistar rats of each sex were given radio labelled carbendazim by gavage as single doses of 3 and 300 mg/kg bw; they were then given repeated daily doses of unlabelled carbendazim for 28 days, followed by a single radio labelled dose. Urine was collected during the first 6 h, after which time the animals were killed. Almost all the metabolites in urine were conjugated with sulfuric acid. Cleavage of these conjugates by ß-glucuronidase-aryl sulfatase released 5-HBC as the only metabolite extractable from water. Mouse urine contained more compounds that remained polar after enzyme treatment than the urine of rats. There was no sex difference. The residual content of carbendazim in the liver was generally lower in rats that were pre-treated with unlabelled carbendazim (Dorn *et al* 1983).

Culik R (1981a) Determination of benomyl/methyl-2-benzimidazole carbamate (MBC) concentrations in maternal blood and in the concepti of rats exposed to benomyl and Benlate by diet. DuPont de Nemours & Co., Haskell Laboratory, Newark, Delaware, USA. Report No. HLR 916-80, dated 23 March 1981. Medical Research Project No. 3501-001 (Expt Date August 1979 - September 1980)

Following a pilot study in which food consumption was measured throughout the dark and light photoperiods, groups of 40 pregnant ChR-CD rats (approximately 180 g bw, age unstated) rats received 10000 ppm benomyl (alone [99.2% purity, batch no. INT-1991-414] or formulated as Benlate [50% benomyl, batch no. INT-1991-442]) in the diet over GD 7-12. A control group of at least 8 dams received plain diet. Food consumption was measured at unspecified intervals, and dams were weighed on GD 3, 7 and at sacrifice. Groups of 8 treated and 2 control dams were sacrificed at 10:00 PM on GD 11 and at 1:00, 4:00 and 8:00 AM on GD 12. Benomyl/carbendazim, 5-HBC and 4-HBC concentrations were measured by an unidentified method in maternal blood and embryos.

During the dosing period, the laboratory was illuminated continuously due to an equipment malfunction. Although the rats continued to consume approximately 80% of their total food intake during the "night" (i.e. between 7:00 PM and 7:00 AM), absolute 24-h food consumption was reduced by approximately 40% in the treated groups and 10% in the controls by comparison with the pilot study animals. Benomyl intake would therefore have been equivalent to approximately 600 mg/kg bw/d.

There was some evidence of circadian variation in the concentrations of benomyl and its metabolites in maternal blood and embryos. The highest mean concentrations of benomyl/carbendazim in maternal blood were present at 4:00 AM, and were 0.6 and 0.23 ppm in the groups receiving benomyl or Benlate, respectively. Benomyl/carbendazim was not detected in embryos (LOD = 0.3 ppm). The highest mean concentrations of 5-HBC in maternal blood were present at 4:00 or 8:00 AM, and were approximately 3.5 - 4.0 ppm in the 2 treated groups. In embryos, the highest mean concentrations of 5-HBC were also detected at 4:00 or 8:00 AM, and were approximately 3.5 and 5.5 ppm in the groups receiving benomyl or Benlate, respectively. 4-HBC was not detected in either maternal blood or embryos (LODs in the respective matrices were 0.03 and 0.08 ppm).

Culik R (1981b) Determination of Benomyl/Methyl-2-benzimidazole Carbamate (MBC), 4-HMBC and 5-HMBC Concentrations in Maternal Blood and in the Concepti of Rats Exposed to Benomyl by Gavage. DuPont Haskell Lab. for Toxicology & Industrial Medicine, Delaware. Report No. 970-80, dated Jan. 29, 1981. Medical Research Project No. 3501-001 (Expt Date Aug. 1979-June 1980)

ChR-CD pregnant rats (approx. 180 g, 91 animals) were given 125 mg/kg bw/d benomyl (99.2% ai, administered in corn oil, prepared daily) by gavage on d 7-16 of gestation. Blood samples were collected 1, 2, 4, 8 and 24 h after dosing on d 7, 12 and 16 of gestation for analysis of benomyl/carbendazim and 5-HBC concentration. Embryos were collected for tissue assays at the same time periods on d 12 and 16.

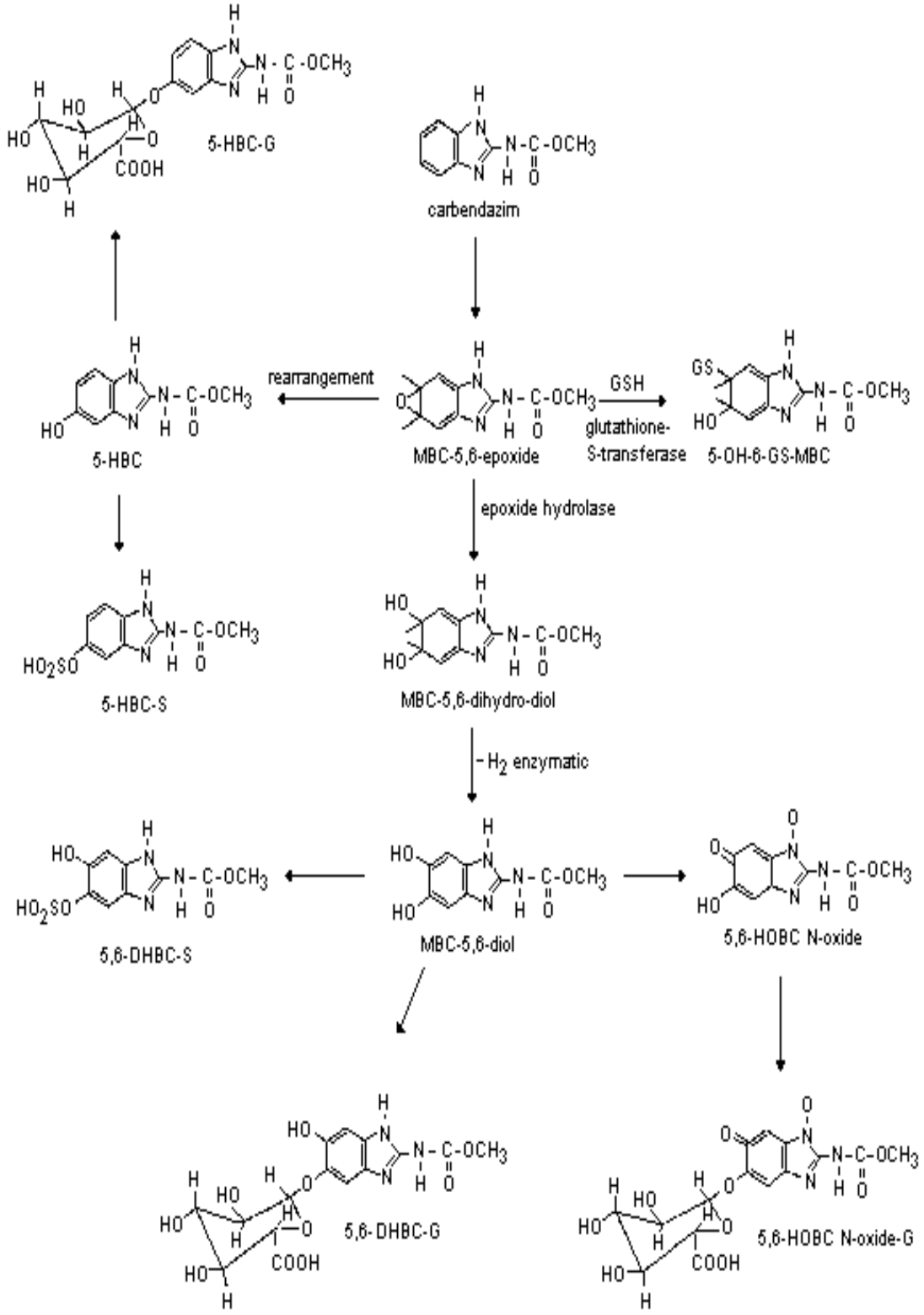
Levels of benomyl/carbendazim in maternal blood were highest one h after gavage on d 7 of gestation (first treatment d) with a mean value of 5.02 ppm. After 6 or 10 consecutive daily treatments, and after one hour following gavage, blood levels were significantly lower, $\leq$ 0.39 ppm. In embryonal tissue, the highest level of benomyl/carbendazim (1.9 ppm for combined sample) was found one hour after dosing on d 12 of gestation, the first d of tissue collection and 6th d of treatment. After 10 consecutive d of treatment, and one h following intubation, the level of benomyl/carbendazim in embryos was 0.13 ppm. The study authors concluded that the increased depletion of benomyl/carbendazim from maternal blood and embryonal tissues following consecutive treatments was suggestive of an increased metabolism pathway or decreased absorption of benomyl. The half-life of benomyl was approx. 45 min in maternal blood, shorter in embryos.

On the other hand, the level of 5-HBC increased somewhat with the number of exposures, with a half-life of approx. 2-3 h in maternal blood and 4-8 h for embryos, indicating a faster metabolism of benomyl and slower depletion of 5-HBC after repeated treatment. On GD 12, one h after gavage, the concentration of 5-HBC ranged up to 2.8 ppm in maternal blood and 2.3 ppm in embryos. On GD 12 and 16, eight h after gavage, the concentration of 5-HBC ranged up to 1.20 ppm in maternal blood and 0.92 ppm in embryos.

Guengerich FP (1981) Enzyme Induction with DuPont Compounds H11, 202-02 and H10, 962-02. Unpublished Report from Vanderbilt University, School of Medicine, Nashville, Tennessee, USA, Prepared for DuPont de Nemours & Co.

The effects of benomyl and carbendazim on hepatic enzymes were studied in male and female SD rats and Swiss albino mice fed diets containing benomyl or carbendazim at a concentration of 0, 10, 30, 100, 300, 1000 or 3000 ppm for 28 d. After sacrifice, liver weights were recorded and microsomal epoxide hydrolase and cytosolic glutathione-S-transferase were monitored in subcellular fractions isolated from the liver. The mean absolute liver weights were elevated in males and females (assumed to be both rats and mice, but not stated in JMPR evaluation) fed 1000 or 3000 ppm carbendazim and in females fed 300 ppm; however, the only significant increase was found in females fed 3000 ppm benomyl. No apparent liver toxicity or effect on bodyweight was observed. Both benomyl and carbendazim induced epoxide hydrolase in male and female rats and in mice fed 1000 or 3000 ppm, and both induced glutathione-S-transferase at 3000 ppm. The level of induction seemed to be slightly greater in females than males. There was no substantial difference in enzyme induction between rats and mice.

Figure 1. Proposed metabolic pathway for carbendazim in rats



3. ACUTE TOXICITY STUDIES

Active Constituent

3.1 Oral and dermal acute toxicity

A summary of the results of acute oral and dermal toxicity studies conducted on technical carbendazim (98% purity) is provided in Table 4.

Table 4 – Summary of acute oral and dermal toxicity

Species	Guidelines	Vehicle	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral acute toxicity					
Wistar rats 5/sex	OECD No. 401	Peanut oil	2000	>2000 No toxic signs or deaths	Kumar (2001a)
Sprague-Dawley rats 5/sex	OECD No. 401	1% methylcellulose	2000	>2000 No toxic signs or deaths	McRae (1997a)
Dermal acute toxicity					
Wistar rats 5/sex	OECD No. 402	Deionised water	2000	>2000 No toxic signs or deaths	Kumar (2001b)
Sprague-Dawley rats 5/sex	OECD No. 402	1% methylcellulose	2000	>2000 No toxic signs or deaths	McRae (1997b)

3.2 Skin irritation

Prakash PJ (2001) Acute dermal irritation/corrosion study with carbendazim technical 98% in New Zealand White rabbits. Toxicology Department, Rallis Research Centre, Bangalore, India. Report number: 3181/1. Report date: 16 August 2001.

Materials and Method: The study was conducted according to the OECD guidelines 404. Three male New Zealand White rabbits (3-4 months old; 2.21-2.32 kg) were acclimatised for 5 days prior to a single application of carbendazim (98% purity) at 0.5 g. The test substance was applied to a shaved skin area of 6 cm^2 by means of a gauze patch. After an exposure period of 4 hours, the patch was removed and skin irritation was evaluated at 1, 24, 48 and 72 hours after patch removal. The degree of irritation was scored according to the Draize Scale. Clinical signs, mortality and bodyweights were recorded daily for 3 days.

Results: There were no signs of toxicity or ill health in any rabbit, and no dermal response to treatment was observed in any animal during the observation period. Carbendazim is not a skin irritant in rabbits.

Parcell BI (1997) Carbendazim technical: Skin irritation to the rabbits. Huntingdon Life Sciences Ltd, Cambridge, England. Report number: 95/970644/SE. Report date: 30 June 1997.

Materials and Method: The study was conducted according to the OECD guidelines 404. Three male New Zealand White rabbits (12 weeks old; 2.4-2.7 kg) were acclimatised for 5 days prior to a single application of carbendazim (99.5% purity) at 0.5 g. The test substance was applied to a shaved skin area of 6 cm^2 by means of a gauze patch. After an exposure period of 4 hours, the patch was removed and skin irritation was evaluated at 1, 24, 48 and 72 hours after patch removal. The degree of irritation was scored according to the Draize Scale. Clinical signs, mortality and bodyweights were recorded daily for 3 days.

Results: There were no signs of toxicity or ill health in any rabbit, and no dermal response to treatment was observed in any animal during the observation period. Carbendazim is not a skin irritant in rabbits.

3.3 Eye irritation

Prakash PJ (2001) Acute eye irritation/corrosion study with carbendazim technical 98% in New Zealand White rabbits. Toxicology Department, Rallis Research Centre, Bangalore, India. Report number: 3182/1. Report date: 16 August 2001.

Materials and Method: The study was conducted according to the OECD guidelines 405. Three male New Zealand White rabbits (3-4 months old; 1.8-2.2 kg) received a single dose of 33 mg of carbendazim (undiluted) into the left conjunctival sac of each animal. The untreated eye served as control. Eyes were examined for irritation and scored according to the Draize scale at 1, 24, 48, 72 hours after treatment.

Results: There were no signs of toxicity or ill health in any rabbit during the observation period. No ocular reaction was observed in any rabbit. Carbendazim is not an eye irritant in rabbits.

Parcell BI (1997) Carbendazim technical: Eye irritation to the rabbits. Huntingdon Life Sciences Ltd, Cambridge, England. Report number: 96/970742/SE. Report date: 30 June 1997.

Materials and Method: The study was conducted according to the OECD guidelines 405. Four male New Zealand White rabbits (13-15 weeks old; 3--3.5 kg) received a single dose of 65 mg of carbendazim (undiluted) into the left conjunctival sac of each animal. The untreated eye served as control. Eyes were examined for irritation and scored according to the Draize scale at 1, 24, 48, 72 hours after treatment.

Results: There were no signs of toxicity or ill health in any rabbit during the observation period. All rabbits had slight conjunctival redness and chemosis one hour after instillation but resolved by 24 hours. Carbendazim is not an eye irritant in rabbits.

3.4 Inhalation toxicity

Jackson CG (1997) Carbendazim technical: Acute inhalation study in rats (4-hour exposure). Huntingdon Life Sciences Ltd, Cambridge, England. Report number: 102/972901/SE. Report date: 18 September 1997.

Materials and methods: Rats (5/sex; 8-9 weeks old, 183-203 g) were acclimatised to standard laboratory conditions for 31 days prior to the start of the study. Food and water were available *ad libitum* except during the exposure period. Carbendazim (99.5% purity), as a respirable particulate aerosol, was administered by nose only exposure for 4 hours at a mean gravimetric chamber concentration of 4280 mg/m^3, which was the highest attainable concentration. The mass median aerodynamic diameters were estimated to be 3.8 μm with a geometric standard deviation of 2.51. Mortality and clinical signs were recorded during exposure, upon removal from the chamber and then once daily for 15 days. Bodyweight was measured prior the study and on days 4, 8 and 15. All animals were sacrificed at the end of the observation period and were subjected to gross pathological examination.

Results: There were no deaths. Signs including exaggerated respiratory movement (laboured respiration and rales), salivation and ruffled fur were seen in all treated animals. All clinical signs resolved one day post exposure in all animals. No macroscopic abnormalities were observed at necropsy.

The LC_{50} was greater than 4280 mg/m^3 for both sexes.

3.5 Skin sensitisation

Sulaiman SM (2001) Skin sensitisation study (Buehler test) with carbendazim technical 98% in guinea pigs. Toxicology Department, Rallis Research Centre, Bangalore, India. Report number: 3183/1. Report date: 16 August 2001.

Materials and methods: The sensitising potential of carbendazim (98% purity) was determined using a modified Buehler method. For induction, Hartley guinea pigs (10/sex) in the test groups were treated with 500 mg of the test substance on the left site of the back for 6 hours under dressing. The treatment was repeated at the same skin site 3 times weekly for 3 weeks (a total of 9 applications). Two weeks after the last induction, a challenge dose of 500 mg of the test substance was applied to the right flank of the animal for 6 hours. Another group of (5/sex) naïve animals remained untreated during the induction phase but received the same challenge application. Mercapto benzothiazole was applied for induction and challenge to another group of 10 male animals as positive control. Dermal reactions were scored at 24 hours after each induction application, and at 24 and 48 hours after the challenge application, according to the grading scale of Magnusson and Kligman. Bodyweights were recorded before application and at termination.

Results: No skin reactions were observed in the control and treated groups. Bodyweights were not affected by treatment. The skin sensitisation rate for the positive control mercapto benzothiazole was 40%. Carbendazim was not a skin sensitiser in guinea pigs in this test.

Products

Acute toxicity studies with a carbendazim-based SC formulation (Shincar 500 SC Fungicide containing 500 g/L carbendazim) are shown below.

3.6 Oral and dermal acute toxicity

A summary of the results of acute oral and dermal toxicity studies conducted using a formulation containing 500 g/L carbendazim is provided in Table 5.

Table 5 – Summary of acute oral and dermal toxicity

Species	Guidelines	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Acute oral toxicity				
Sprague-Dawley rats 5/sex	OECD No. 401	2000	>2000 No toxic signs or deaths	McRae (1996a)
Acute dermal toxicity				
Sprague-Dawley rats 5/sex	OECD No. 402	2000	>2000 No deaths. Slight dermal irritation in two animals but resolved by day 3	McRae (1996b)

3.7 Skin irritation

Parcell BI (1995) Carbendazim 500 g/L SC: Skin irritation to the rabbits. Huntingdon Life Sciences Ltd, Cambridge, England. Report number: 31a/952653/SE. Report date: 12 December 1995.

Materials and Method: The study was conducted according to the OECD guidelines 404. Three male New Zealand White rabbits (11-14 weeks old; 2.4-3.3 kg) were acclimatised for 5 days prior to a single semi-occlusive application of 0.1 mL carbendazim (SC 50%). The test substance was applied to a shaved skin area of 6 cm^2 by means of a gauze patch. After an exposure period of 4 hours, the patch was removed and skin irritation was evaluated at 1, 24, 48 and 72 hours after patch removal. The degree of irritation was scored according to the Draize Scale. Clinical signs, mortality and bodyweights were recorded daily for 3 days.

Results: There were no signs of toxicity or ill health in any rabbit, and no dermal response to treatment was observed in any animal during the observation period. Carbendazim SC 50% is not a skin irritant in rabbits.

3.8 Eye irritation

Parcell BI (1995) Carbendazim 500 g/L SC: Eye irritation to the rabbits. Huntingdon Life Sciences Ltd, Cambridge, England. Report number: 32a/952767/SE. Report date: 12 December 1995.

Materials and Method: The study was conducted according to the OECD guidelines 405. Three male New Zealand White rabbits (13-15 weeks old; 2.8--3.4 kg) received a single dose of o.1 mL of the formulation into the left conjunctival sac of each animal. The untreated eye served as control. Eyes were examined for irritation and scored according to the Draize scale at 1, 24, 48, 72 hours after treatment.

Results: There were no signs of toxicity or ill health in any rabbit during the observation period. All rabbits had slight conjunctival redness one hour after instillation but resolved by 24 hours. The SC formulation containing 500 g/L carbendazim is not an eye irritant in rabbits.

3.9 Inhalation toxicity

You Y (1999) Acute inhalation toxicity study of Carbendazim SC 50% in rat. Supervision and Test Centre for Pesticide Safety Evaluation, Liaoning Province, China. Report number: R9919104S0. Report date: 29 March 1999.

Materials and methods: Rats (5/sex/dose; 8-9 weeks old, 180-220 g) were acclimatised to standard laboratory conditions for 31 days prior to the start of the study. Food and water were available *ad libitum* except during the exposure period. Animals were exposed to carbendazim (SC 50%) for 2 hours at a mean gravimetric chamber concentrations of 0, 215, 464, 1000 or 2150 mg/m^3. Mortality and clinical signs were recorded during exposure, upon removal from the chamber and then once daily for 14 days. All animals were sacrificed at the end of the observation period and were subjected to gross pathological examination.

Results: There were no deaths. Animals were lethargic when during exposure period but resolved one day post exposure. No macroscopic abnormalities were observed at necropsy.

The LC_{50} was greater than 2150 mg/m^3 for both sexes.

3.10 Skin sensitisation

Allan S (1995) Carbendazim 500 g/L SC: Skin sensitisation study (Buehler test) in guinea pigs. Huntingdon Life Sciences Ltd, Cambridge, England. Report number: 32a/952767/SE. Report date: 12 December 1995. Report number: 33a/952864/SS. Report date: 12 December 1995.

Materials and methods: The sensitising potential of carbendazim SC 50% was determined using a modified Buehler method. For induction, Hartley guinea pigs (20/sex) in the test groups were treated with 0.5 mL of the test substance on the left site of the back for 6 hours under dressing. The treatment was repeated at the same skin site 3 times weekly for 3 weeks (a total of 9 applications). Two weeks after the last induction, a challenge dose 0.5 mL of the test substance was applied to the right flank of the animal for 6 hours. Another group of (10/sex) naïve animals remained untreated during the induction phase but received the same challenge application. Dermal reactions were scored at 24 hours after each induction application, and at 24 and 48 hours after the challenge application, according to the Buehler grading scale. Positive control data (Buehler test) for formalin from Huntingdon Laboratory was provided.

Results: No skin reactions were observed in the control and treated groups. Carbendazim SC 50% was not a skin sensitiser in guinea pigs in this test.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1 Dermal Application

Fave A (1981) 21-day percutaneous toxicity study in rabbits with carbendazim (WNT 80/228). IFREB Domaine des Oncins< L'Arbresle, France. Report No. 007210. Report date: 30 June 1981.

No GLP and QA statement

Methods: New Zealand White rabbits (5/sex/group, 12 weeks old; 2-2.75 kg bodyweight) were dosed with carbendazim suspended in water via the dermal route at 0, 400, 2000 or 10000 mg/kg bw/d for 21 days (5 applications/week, 1 application/d, 6 h/d). Control rabbits received water only. Observations for mortalities and clinical signs were made daily, with detailed clinical examinations. Bodyweight and food consumption was recorded twice a week. At the end of the treatment period, surviving rabbits were necropsied. The following haematology parameters were analysed before treatment and at termination: packed cell volume, erythrocyte count, white blood cell cont, thrombocyte count and differential count. The following clinical chemistry parameters were analysed before treatment and at termination: calcium, potassium, bilirubin (total and direct), total protein, cholesterol, glucose, LDH, ALT, AST, ALT and urea.

The following organs were weighed: brain, kidneys, heart, spleen, liver, pituitary, adrenals, and thyroid. These organs were examined for any gross abnormalities. Any tissues of any animals showing gross lesions were evaluated histologically. Note that testes were not examined.

Results: There were no treatment-related effects on mortalities, clinical signs or bodyweight and food consumption. No treatment-related changes were seen in haematological and clinical chemistry parameter. There were no apparent treatment-related effects on organ weights or microscopic alterations.

4.1 Oral Application

The following short-term oral studies in rats and dogs are reproduced from a JMPR report (2005).

Scholz & Weigand (1972) W17411 = 2-carbomethoxyaminobenzimidazol. Toxikologische Prufung. Range finding test (30 Tage) an Ratten. Unpublished Doc. No. A00011 from Hoechst Pharma Forschung Toxikologie. Submitted to WHO by Bayer CropScience, Monheim, Germany.

In a dose range-finding study, groups of Wistar rats (10/sex/group) received diets containing carbendazim (purity not indicated) at a concentration of 0, 80, 400, 2000, 10000 or 50000 ppm (equivalent to 0, 8, 40, 200, 1000, and 5000 mg/kg bw/d) for 30 days. A decrease in

bodyweight and weight gain was observed at 10000 and 50000 ppm. At 50000 ppm, feed consumption was reduced, and eight males and eight females died with severe emaciation. Leukopenia, siderosis in liver and kidneys and arrest of spermatogenesis were observed in surviving animals at 10000 ppm. Inhibition of spermatogenesis was also observed in three animals at 10000 ppm. The NOEL was 2000 ppm (equivalent to 200 mg/kg bw/d) based on reduced bodyweight gain and inhibition of spermatogenesis at 10000 ppm and above.

Sherman, H. and Krauss, W.C. (1966). Acute oral test (carbendazim). Unpublished report from E.I. Du Pont de Nemours and Co., Inc., Haskell Laboratory, Newark, Delaware.

Groups of six male Sprague Dawley rats were given carbendazim by gavage at a dose of 0, 200, 3400, or 5000 mg/kg bw/d, five times per week for two weeks. Two rats at 3400 mg/kg bw per day died. At all treated doses, gross and microscopic evidence of adverse effects on testes and reduction or absence of sperm in the epididymides was seen. The testes were small and discoloured, with tubular degeneration and evidence of aspermatogenesis. At 3400 mg/kg bw/d, there were also morphological changes in the duodenum (oedema and focal necrosis), bone marrow (reduction in the blood-forming elements), and liver (decrease in large, globular-shaped vacuoles).

Hunter, B., Batham, P. & Newman, A.J. (1973a) Carbendazim oral toxicity to rats for 2 weeks. Unpublished report from Huntingdon Research Centre, United Kingdom. Submitted to WHO by Hoechst AG, Frankfurt, Germany.

Groups of 10 male Sprague-Dawley rats were given carbendazim by gavage at a dose of 0, 10, 20, 30, or 40 mg/kg bw/d for two weeks. At the high dose, liver weights were increased. There were no histopathological findings and no effects on spermatogenesis, on cellularity, or on the incidence of mitosis.

Til, H.P., Leegwater, D.C. & Feron, V.J. (1971) Tentative (28-day) feeding study with W17411 in beagle dogs. Unpublished report No. R3659, Doc. No. A00015, from Central Institute for Nutrition and Food Research (TNO), the Hague, Netherlands. Submitted to WHO by Bayer CropScience, Monheim, Germany.

Groups of two male and two female juvenile beagle dogs were fed carbendazim (purity not indicated) at a dietary concentration of 0, 500, or 2500 ppm (equal to 0, 19–21, and 96–99 mg/kg bw/d, depending on sex) for 28 days. Bodyweight, development, feed consumption, and overall health were not affected by treatment. Liver weights were increased in females at 500 and 2500 ppm (161% and 216% higher, respectively). ALT and ALP activity was increased in males at 2500 ppm. Pathological changes were confined to the liver at 2500 ppm. In males, effects were mainly characterized by peliosis-like changes, bile-duct proliferation, accumulations of reticulo-endothelial cells, and periportal cellular infiltration. In females, the changes consisted of greatly enlarged hepatocytes with a watery pale-staining cytoplasm. The NOEL was 500 ppm (equivalent to 19-21 mg/kg bw/d) based liver toxicity at 2500 ppm.

5. SUBCHRONIC TOXICITY STUDIES

Hunter B (1973b) BMC toxicity in rats during dietary administration for 13 weeks followed by a recovery period of 6 weeks. Huntingdon Research Centre, Huntingdon, England. Report no. BSF28/73533. Report date: 26 October 1973.

Methods: Sprague-Dawley rats (80/sex/group, average bodyweight 2.5 g; 4 weeks old) were fed carbendazim in the diet at 0, 50, 150, 450 or 1350 ppm (equivalent to approximately 0, 2.5, 12.5, 22.5 or 67.5 mg/kg bw/d) for 13 weeks followed by a recovery period of 6 weeks. Animals were examined daily for behaviour and for clinical signs of toxicity, and weekly for bodyweight changes. Haematology, clinical chemistry and urinalysis were performed at weeks 4, 8, 12 and 18. After 13 weeks of treatment, 20 males and 20 females from each group were killed. On completion of a further 6 weeks recovery period, all surviving animals were killed. The following tissues were weighed and examined macro- and microscopically: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid and uterus.

Results: The only treatment-related effects were increased in relative liver weight in both sexes at 1350 ppm (8% and 14% for males and females, respectively) but returned to normal following the 6-week recovery period. The NOEL was 22.5 mg/kg bw/d.

Scholz & Schultes (1973) Report on a subchronic feeding experiment (93 days) with technical active substance HOE 17411 OF. Unpublished Doc. No. A00409 from Hoechst Pharma Fo. To., FRG. Submitted to WHO by Bayer CropScience, Monheim, Germany.

This study was derived from a JMPR report (2005) and was not independently evaluated.

Groups of 20 male and 20 female Wistar rats (20/sex/dose) received diets containing carbendazim (purity not indicated) at a concentration of 0, 80, 400, 2000 or 10000 ppm (equivalent to 0, 6.5, 32, 163, and 780 mg/kg bw/d in males and 0, 6.9, 36, 174, and 847 mg/kg bw/d in females) for 93 days. Half of the animals in each group were sacrificed, and the remaining rats were fed untreated diet for a 12- to 14-day recovery period. Bodyweight was reduced at 10000 ppm, but no effects were observed on feed consumption. No clinical signs of toxicity were observed. After 93 days of treatment, relative liver weights were increased in females at 400 ppm and in males at 2000 ppm. These changes were not evident after the recovery period. No histopathological lesions or liver enzyme changes were noted. One male at 10000 ppm had small testes and atrophy of the seminiferous tubuli. The NOEL was 2000 ppm (equivalent to 163 mg/kg bw/d) based on reduced bodyweight and the effects in the testes in one male at 10000 ppm.

Til HP et al (1972) Sub-chronic (90 day) toxicity study with W17411 in beagle dogs. Unpublished report from Central Institute for Nutrition and Food Research (TNO), The Hague, Netherlands. Submitted to WHO by Hoechst AG, Frankfurt, Germany.

This study is reproduced from the JMPR report on carbendazim (2005).

Groups of beagle dogs (4/sex/dose) were given carbendazim in the diet at 0, 100, 300, or 1000 ppm for 13 weeks (equivalent to 0, 2.5, 7.5 or 25 mg/kg bw/d). The highest level was increased to 2000 ppm (equivalent to 50 mg/kg bw/d) after six weeks of treatment. Bodyweight, haematological, blood chemistry and urine measurements, and liver and kidney function tests were performed periodically. The animals were examined grossly and

microscopically at the end of the study. There were no reported treatment-related effects on clinical behaviour, bodyweight, food consumption, haematological parameters, kidney or liver function. Clinical chemistry was normal, except for a slight decrease in albumin in males at the mid- and high doses at 12 weeks. Urinalysis showed normal values, except for a high bacterial count in females at the high dose at week 13. The blood clotting time was slightly reduced in dogs at the high dose at week 12. There were slight increases in relative liver and thyroid weights and a decrease in relative heart weights in the group at 2000 ppm. No microscopic changes that could be associated with treatment were observed in these or other organs. The NOEL was 300 ppm, equivalent to 7.5 mg/kg bw/d, on the basis of minor changes in clinical chemistry and organ weights.

6. CHRONIC TOXICITY STUDIES

Beems, R.B et al (1976) Carcinogenicity study with carbendazim (99% MBC) in mice. Unpublished report from Central Institute for Nutrition and Food Research (TNO), The Hague, Netherlands. Submitted to WHO by Hoechst AG, Frankfurt, and BASF AG, Ludwigshafen, Germany.

No GLP or QA statement

Methods: Groups of Swiss mice (100/sex/group) were given carbendazim in the diet at 0, 150, 300, or 1000 ppm for 80 weeks (equivalent to 0, 23, 45 or 150 mg/kg bw/d). The highest dose was increased to 2000 ppm (equivalent to 300 mg/kg bw/d) at week 4 and to 5000 ppm (equivalent to 750 mg/kg bw/d) at week 8 for the remainder of the study. Animals were examined daily for behaviour and for clinical signs of toxicity, and biweekly for palpable masses and regularly for bodyweight changes. All animals were examined grossly, liver and kidney weights were recorded, and tissues were examined microscopically.

Results: Survival was unaffected by treatment. There were no treatment-related effects on general conditions or bodyweight. At termination of the study, 70% of males and 80% of females were still alive. The relative liver weights of males and females at the high dose were significantly higher (20-25% higher). No carcinogenic effect was observed in any tissues. The NOEL was 300 (equivalent to 45 mg/kg bw/d) based on increased relative liver weight at the higher dose. Carbendazim was not a carcinogen in this strain of mouse.

Wood, C.K. (1982) Long-term feeding study with 2-benzimidazole carbamate, methyl ester (INE-965) in mice. Unpublished report from E.I. DuPont de Nemours and Co, Inc., Haskell Laboratory, Newark, Delaware, USA. Report number: 3207-001. Report date: 26 January 1982.

No GLP and QA statement

Methods: Groups of CD-1 mice (80/sex/group), aged 6-7 weeks, were given carbendazim (purity, 99%) in the diet at 0, 500, 1500, or 7500 ppm for two years (equivalent to 0, 75, 225 or 1125 mg/kg bw/d). The highest dose was reduced to 3750 ppm (equivalent to 563 mg/kg bw/d) for the males after 66 weeks because of increased mortality (62 for controls and 32 at 7500 ppm); females, however, received 7500 ppm throughout the study. Treatment affected mortality in male mice, and those at the high dose were sacrificed at week 73 because only 23 were still alive. Only nine males at 1500 ppm survived to week 104, whereas 18 male controls

were still alive at that time. Females had no similar increase in mortality. Animals were examined daily for behaviour and for clinical signs of toxicity, and biweekly for palpable masses and regularly for bodyweight changes. All animals were examined grossly, liver and kidney weights were recorded, and tissues were examined microscopically.

Results: Mortality in male mice and those at the high dose were sacrificed at week 73 because only 23/80 still alive. Females had no similar increase in mortality. There were no effects on bodyweight or food consumption. Clinical parameters were similar for all treated and control groups, and haematological parameters were unaffected. Relative liver weights were increased in females at 1500 and 7500 ppm (25-28% higher). A significant hepatotoxic effect was seen in male mice at 1500 and 7500 ppm, as demonstrated by centrilobular hypertrophy, necrosis, and swelling. There was no increase in the frequency of hepatocellular adenomas, as they occurred at equal frequency in control and treated groups. There was a significant increase in the incidence of hepatocellular carcinomas, but only at 1500 ppm; however, too few males at the high dose survived to 17 months (510 days) to support the conclusion that there is no oncogenic effect at that dose. In addition, the high mortality rate in male controls feather hampered the interpretation of results. Histopathological analysis of the hepatocellular tumours in the test animals showed no difference from controls, and the median latent period for development of these hepatocellular carcinomas showed no significant decrease in treated animals. No carcinogenic effect was observed in tissues other than the liver (Table 6). The NOEL was 500 (equivalent to 75 mg/kg bw/d) based on effects on the liver at higher doses.

Table 6 : Hepatic tumour incidence in CD-1 mice fed carbendazim

Occurrence	Control	500 ppm	1500 ppm	7500 ppm
	Male			
Hepatocellular carcinomas	13/80 (16%)	20/80 (25%)	28/80 (28%)	ND*
Median time to discovery of tumours	633	697	651	ND*
	Female			
Hepatocellular carcinomas	1/80 (1%)	9/79 (11%)	21/80 (26%)	15/80 (18%)
Median time to discovery of tumours	732	706	689	753

*This group was terminated after 516 days of treatment due ti high rate of mortality.

Donaubauer, H. et al (1982) Repeated dose (24 month) feeding study for determination of the carcinogenic effect of HOE 17411 OFAT204 (carbendazim) in mice. Unpublished report from Hoechst AG, Pharmaceuticals Research, Toxicology Section, Frankfurt, Germany. Report number: 643/83. Report date: 13 October 1982.

No GLP and QA: QA statement

Methods: Groups of NMRKf mice (100-120/sex/group) were given carbendazim in the diet at 0, 50, 150, 300, or 1000 ppm (estimated to be equivalent to 0, 7.5, 22.5, 45 or 150 mg/kg bw/d respectively) for 96 weeks. The highest dose was increased to 2000 ppm (equivalent to 300 mg/kg bw/d) at week 4 and to 5000 ppm (equivalent to 750 mg/kg bw/d) at week 8 for the remainder of the study. Animals were examined daily for mortality, behaviour and general

condition and biweekly for bodyweight, food and water consumption. Gross necropsy was performed on all animals, liver and lung weights were recorded, and all organs and tissues were examined microscopically. An interim sacrifice was conducted of 20 males and 20 females in the control and highest dose groups at 18 months.

Results: There were no treatment-related effects on mortality, behaviour, bodyweight gain, and food or water consumption. By 22 months, 24-31% of the males and 37-52% of the females had died. As there was no difference between the treated and control groups, it was concluded that mortality was not influenced by carbendazim. At 18 and 22 months, the relative liver weights of both male and female mice at 5000 ppm were increased (17-23%). At the 18 month interim sacrifice, macroscopic and microscopic examination revealed treatment-related effects on the liver, with all animals having centrilobular hypertrophy, single-cell necrosis, mitotic cells, and pigmented Kupffer cells. The tissues of the remaining 100 males and 100 females at 5000 ppm, examined at 22 months, showed marked hypertrophy (greater than in animals treated for 18 months), clear-cell foci, mitosis, inclusion bodies in enlarged cell nuclei, multiple cell necrosis, and a greenish-yellow pigment in Kupffer cells. Neoplastic nodules (adenomas), carcinomas, fibrosarcomas, and other tumorigenic responses in the liver were similar among groups. There was no effect on the incidence or time of onset of tumours, and the total number of benign and malignant tumours was comparable among groups. It was concluded that carbendazim was not a carcinogen in this strain of mouse. The NOEL was 300 ppm (45 mg/kg bw/d) based on liver effects at 5000 ppm.

Sherman, H. (1972) Long-term feeding studies in rats and dogs with 2-benzimadazole carbamic acid, methyl ester (INE-965) (50% and 70% MBC wettable powder formulations). Unpublished report from E.I. DuPont de Nemours and Co., Inc., Haskell Laboratory, Newark, Delaware, USA. Report number: 195-72. Report date: 25 May 1972.

(Note. This study consisted of three parts: long-term rat study, long-term dog study and rat reproduction study. Each part has been written up in the respectively appropriate section.)

No GLP and QA statement

Methods: Groups of SD rats (36/sex/dose) were given carbendazim (purity, 50-70%) in the diet for 104 weeks at 0, 100, 500, 2500 (increased to 10000 ppm after 20 weeks), or 5000 ppm (equivalent to 0, 5, 25, 125/500 or 250 mg/kg bw/d). Bodyweight and food consumption were recorded weekly for the first year and twice a month thereafter. Behavioural changes and mortality were observed daily. Haematological, urinary, and selected clinical chemical examinations were performed periodically. After one year, each group was reduced to 30 male and 30 female rats by interim sacrifice for gross and microscopic examinations. At the end of the study, all surviving animals were sacrificed, and tissues and organs were examined grossly. Microscopic examinations were conducted on all tissues and organs from the controls and animals at 2500/10000 ppm, the livers of animals at 100 and 500 ppm, and the livers, kidneys, testes, and bone marrow of animals at 5000 ppm.

Results: The toxic effects were restricted to the highest dose. Bodyweight gain was depressed in both sexes. Food consumption was similar in all groups. Reduced erythrocyte counts and haemoglobin and hematocrit values were seen in females after 9-24 months and in males after 24 months. There were no compound-related clinical manifestations of toxicity and no effects on urinary parameters. ALP and ALT activities varied throughout the study in animals at the highest dose, but there was no consistent dose-response relationship. Histopathological examination of the livers showed no treatment-related effects. Males had an

increased incidence of diffuse testicular atrophy (8/30 or 27% versus 2/28 or 7%) and prostatitis (6/30 or 20% versus 2/28 or 10%) compared to controls. The NOEL was 2500 ppm (equivalent to 125 mg/kg bw/d).

Til, H.P et al (1976a) Combined chronic toxicity and carcinogenicity study with carbendazim in rats. Unpublished report from Central Institute for Nutrition and Food Research (TNO), The Hague, Netherlands. Submitted to WHO by BASF AG, Ludwigshafen, and Hoechst AG, Frankfurt, Germany. Report number: R 5133. Report date: September 1976.

No GLP and QA statement

Methods: Groups of Wistar rats (60/sex/dose) were given carbendazim (purity, 99%) in the diet at 0, 150, 300, or 2000 ppm for two years (equivalent to 0, 7.5, 15 or 100 mg/kg bw/d). The dose of 2000 ppm was increased to 5000 ppm (equivalent 250 mg/kg bw/d) after one week and to 10000 ppm (equivalent 500 mg/kg bw/d) after two weeks for the remainder of the study. Animals were examined daily for clinical signs of toxicity. Bodyweight and food consumption were measured regularly throughout the study. Haematological parameters, clinical chemistry, and urinalysis were conducted periodically. All animals were subjected to complete gross necropsy, and selected organs were weighed. Tissues from 20 male and 20 female rats in the control and high-dose groups were examined microscopically, and all tumours and gross abnormalities were examined histologically.

Results: There were no treatment-related effects on mortality, and survival at termination of the study was similar in all groups. No differences in clinical signs, bodyweight gain or food consumption were seen between test groups and control animals. The results of urinalyses were comparable among the groups. The haemoglobin level was depressed in females at 10000 ppm females at weeks 26, 52, and 103 (5, 5 and 12% lower respectively). AST activity was decreased in high-dose males at termination of the study (25% lower), but not in females. Relative liver weights were increased in both sexes at 10000 ppm (10-15%). There were no histological differences between control and treated groups. The number of tumours was comparable among all groups, and no compound-related oncogenic effects were reported. The NOEL was 300 ppm (equivalent to 15 mg/kg bw/d), on the basis of changes in liver weights and minor biochemical changes at 10000 ppm.

Reuzel, P.G.J., Hendriksen, C.F.M & Til, H.P (1976) Long-term (two-year) toxicity study with carbendazim in beagle dogs. Unpublished report from the Central Institute for Nutrition and Food Research for BASF. Submitted to WHO by E.I. DuPont de Nemours and Co., Wilmington, Delaware, USA. Report number: R5023. Report date: June 1976.

No GLP and QA: QA statement

Methods: Groups of beagle dogs (4/sex/dose), aged 22-27 weeks, were given carbendazim in the diet at 0, 150, 300, or 2000 ppm for 104 weeks (equivalent to 0, 3.8, 7.5, or 50 mg/kg bw/d). After 33 weeks, the dose of 2000 ppm was increased to 5000 ppm (equivalent to 125 mg/kg bw/d). The dogs were examined daily for clinical signs of toxicity and altered behaviour; bodyweight and food consumption were recorded regularly throughout the study. Haematological examinations, blood chemistry (including liver and kidney function tests), and urinary measurements were conducted periodically. After 104 weeks, the dogs were sacrificed, the organs were weighed and the tissues were examined grossly and microscopically.

Results: One female at the high dose was killed in a moribund state after week 36. Bodyweight gain and food consumption was comparable in all groups. ALP activity was increased at the high dose throughout the study (40% higher). All other haematological parameters, blood chemistry and urinalysis were comparable with those of the controls. Liver and thyroid weights were significantly increased at the high dose (32% and 24% higher respectively), but there were no microscopic changes in these organs that were related to treatment. An increased incidence of prostatitis was seen in high-dose males in comparison with controls (3/4 versus 1/4). One male at that dose also had interstitial mononuclear inflammatory cell infiltrates and atrophic tubules of the testes. The NOEL was 300 ppm (equivalent to 7.5 mg/kg bw/d) on the basis of changes in prostate and testes as well as minor biochemical changes at 5000 ppm.

Sherman, H. (1972) Long-term feeding studies in rats and dogs with 2-benzimadazole carbamic acid, methyl ester (INE-965) (50% and 70% MBC wettable powder formulations). Unpublished report from E.I. DuPont de Nemours and Co., Inc., Haskell Laboratory, Newark, Delaware, USA. Report number: 195-72. Report date: 25 May 1972.

(Note. This study consisted of three parts: long-term rat study, long-term dog study and rat reproduction study. Each part has been written up in the respectively appropriate section.)

No GLP and QA statement

Methods: Groups of beagle dogs (4/sex/dose), one to two years of age, were given carbendazim (purity, 53%) in the diet at 0, 100, 500, or 2500 ppm for two years (equivalent to 0, 2.5, 12.5 or 63 mg/kg bw/d). Food consumption and bodyweights were measured weekly, and animals were examined daily for clinical signs of toxicity. Haematological, biochemical, and urinary examinations were performed periodically throughout the study. After one year, one male and one female in the control and 500 ppm groups were killed. At the end of the study, the organs were weighed, and gross and histopathological examinations were performed.

Results: Three males at the high dose were sacrificed after 22 and 42 weeks because of poor nutrition. Haematological and urinary values were unaffected by treatment. The dogs at 500 ppm had increased levels of cholesterol, BUN, total protein, and serum ALT. Swollen, vacuolated hepatic cells and marginal proliferation of the portal triads with cellular infiltration were observed in one dog at 500 ppm and was sacrificed after one year. The biochemical evidence of an effect on the liver was corroborated by the finding at terminal sacrifice of hepatic cirrhosis, swollen, vacuolated hepatic cells, and mild chronic hepatitis at 500 ppm or higher. There were no effects on organ weights. Diffuse testicular atrophy and inhibition of spermatogenesis were observed in two of four males at 100 ppm but not at 500 or 2500 ppm. As similar effects were not seen at the next higher dose, these findings were not considered as being treatment-related. The NOEL was 100 ppm (equivalent to 2.5 mg/kg bw/d), based on the effects on the liver at 500 ppm.

7. REPRODUCTION STUDIES

Gray LE et al (1990) Carbendazim-induced alterations of reproductive development and function in the rats and hamster. Fund. Appl. Toxicol. 15: 281-297.

Materials and methods: In this study, a total of 88 Long-Evan hooded rats were treated with carbendazim orally by gavage at 0, 50, 100, 200 or 400 mg/kg bw/d. The groups consisted of 24 control animals (12/sex) and 16 animals (8/sex) in each of the four treated groups. Treatment was initiated at weaning (day 21). In females, dosing was continued through post-partum day 20, while males were dosed daily from weaning until necropsy. Male and female rats from the same group were paired for 16 days, starting at 84 days of age. Males were necropsied at 104-106 days of age. Body, testes, liver, kidney, adrenal, epididymis and pituitary were weighed. One testis was fixed for histological examination. Caudal sperm morphology and motility was assessed. At 35 days of age, F1 pups were randomly selected for fertility assessment. Breeding was monitored continuously for four months using 20 pairs of control and 22 pairs of carbendazim-treated at 100 mg/kg bw/d offspring. F2 pups were counted and removed on post-natal day 1. At 5 months of age, the males of F1 pups were necropsied and the liver, kidney, adrenals, testis, cauda epididymis, pituitary and seminal vesicles were weighed. One testis was fixed for histological examination.

A total of 54 Syrian hamsters were also treated with carbendazim by gavage at 0 (15/sex) or 400 mg/kg bw/d (12/sex). Treatment was initiated at weaning (day 22). In females, dosing was continued up to parturition, while males were dosed daily from weaning until necropsy. At 85 days of age, males were necropsied and body, testes, liver, kidney, adrenal, epididymis and pituitary were weighed. One testis was fixed for histological examination. Caudal sperm morphology and motility was assessed.

Results: There were no unscheduled deaths. At 200 and 400 mg/kg bw/d, the number of pregnant female rats was reduced and it was subsequently determined that these females had been paired with males with severe testicular atrophy and very low sperm counts. Female rats that became pregnant in these two groups had lower foetal viability. There were no viable litters at 400 mg/kg bw/d and only three at 200 mg/kg bw/d. One of the three litters at 200 mg/kg bw/d and 2/7 at 100 mg/kg bw/d had malformed pups (visibly hydrocephalic).

Testis and cauda epididymal weights were reduced by about 40% compared to control at 200 and 400 mg/kg bw/d (Table 7). Histologically, 2/8, 3/8 animals at 50 and 100 mg/kg bw/d had mild testicular atrophy, respectively. At 200 and 400 mg/kg bw/d, 5/8 and 6/8 animals had moderate/severe testicular atrophy, respectively. Testicular sperm counts were reduced at 200 and 400 mg/kg bw, while cauda epididymal sperm count was reduced in all treated groups in a dose-related manner. Semen quality and sperm morphology were altered at all doses, while sperm motility was reduced at 200 and 400 mg/kg bw/d.

All of the foetuses were resorbed at 400 mg/kg bw/d and most of the pups died at 200 mg/kg bw/d.

Table 7: Reproductive effects in rats (F0)

	Dose (mg/kg bw/d)				
	0	**50**	**100**	**200**	**400**
No. of pairs and mated	11	8	8	7	7
No. of pregnant animals	11	8	8	3*	4*
No. of live litters	9	8	7	3	0
No. of dams died	1	0	1	0	0
No. of litters resorbed	1	0	0	0	4*
No. of malformed litters	0	0	2	1	0
Maternal bodyweight gains (g)	125	119	115	89*	41***
Testicular head sperm count (millions)	199	185	179	63	61
Cauda epididymal sperm count (million)	141	113**	101*	17***	11***
Motility estimate (%)	58	58	54	16***	13***
No. males with debris in samples	1/12	8/8**	8/8**	8/8**	8/8**
No. males with broken sperm	0/12	5/8**	4/8**	7/8**	8/8**
Sperm with degenerate tails (%)	0.8	0.8	2	9**	6**
Sperm with misshapen head (%)	0.8	0.8	2	25**	18**

*p < 0.05; **p < 0.01; ***p < 0.001

For F1 rats that were indirectly exposed to carbendazim, the growth, viability and reproductive performance was not affected at 100 mg/kg bw/d.

The reproductive performance in hamsters was much less affected by carbendazim at 400 mg/kg bw/d. Males had slightly lower sperm counts in the testis (17% lower) and cauda epididymis (16%) but this did not reduce fertility.

No NOEL was established based on effects sperm production and morphology observed in rats at the lowest dose.

Cummings AM et al (1990) Effects of methyl benzimidazole carbamate during early pregnancy in the rat. Fund. Appl. Toxicol. 15:528-535.

Materials and methods: In this literature report, a study was designed to assess potential maternal effects of carbendazim during early pregnancy to distinguish maternal from embryotoxic effects of the carbendazim, and to differentiate between early pregnancy failure and late embryonic loss. Carbendazim was administered to rats by gavage at 0, 25, 50, 100, 200, 400, and 1000 mg/kg bw/d during Days 1 through 8 of pregnancy (Day 0 = sperm positive). A range of maternal and embryonic parameters was assessed following euthanasia on Day 9, including the number of implantation sites, bodyweight gain, uterine weight, implantation site size, and serum ovarian and pituitary hormones. In a separate experiment, pregnant rats were administered 0 or 400 mg/kg bw/d during Days 1-8, received bilateral uterine decidual induction on Day 4, and were killed on Day 9 at which time the decidual cell response was evaluated as a measure of uterine competency.

Results: Increased resorptions were observed at higher doses but the increases were not dose-related (1.8, 1.6, 0.8, 3.9, 3, 5 and 0.3 for the seven doses, respectively). This was considered to be incidental to treatment. Other parameters were not affected by treatment. When administered during pseudopregnancy, 400 mg/kg/day MBC partially reduced uterine decidual growth but affected no other parameter. The results showed that doses of carbendazim which are teratogenic when administered to rats in late pregnancy do not produce pregnancy failure.

Carter, S.D, Hess, R.A. & Laskey, J.W. (1987) The fungicide methyl 2-benzimidazole carbamate causes infertility in male Sprague- Dawley rats. Biol. Reprod. 37:709-718.

Materials and methods: In this literature report, a serial breeding technique was used to evaluate the fertility of male SD rats after exposure by gavage to 10 daily doses of 400 mg/kg bw/d carbendazim. Males, 90 days old and proven to be fertile, were bred with a new female each week, starting on the third day of treatment and continuing for 32 weeks after the last day of treatment. Twelve days after each breeding period, the females were killed, their uteri were examined for resorptions, and the numbers of dead and viable foetuses were determined. All males were killed 35 weeks after treatment, and testicular tissue was prepared for histopathological examination by vascular perfusion.

Results: The fertility of treated males (as indicated by the number of pregnant females) was depressed during the first week after treatment: 10 of the 24 treated males failed to induce a pregnancy, as compared with no failure in the control group. By the fifth week after treatment, 16 of the 24 carbendazim-treated males were infertile. Of these, four recovered fertility after being infertile for 5-11 consecutive breeding periods, but the other 12 did not recover during the remainder of the 32-week period after treatment. Histological examinations of testicular sections of the latter animals 245 days after treatment revealed severe seminiferous tubular atrophy (> 85% of tubules were atrophic), often with epithelium containing only Sertoli cells, surrounded by a thickened basement membrane. The lumina of < 2% of the tubules contained spermatozoa. The seminiferous tubules of the treated males that recovered fertility had various contents of atrophic tubules (13-85%) 245 days after treatment.

Sherman, H. (1972) Long-term feeding studies in rats and dogs with 2-benzimadazole carbamic acid, methyl ester (INE-965) (50% and 70% MBC wettable powder formulations). Unpublished report from E.I. DuPont de Nemours and Co., Inc., Haskell Laboratory, Newark, Delaware, USA. Report number: 195-72. Report date: 25 May 1972.

(Note. This study consisted of three parts: long-term rat study, long-term dog study and rat reproduction study. Each part has been written up in the respectively appropriate section.)

Materials and methods: ChR-CD rats (16/sex/dose; 30 do, unspecified bodyweight) were fed diets containing 1% corn oil supplemented with 0, 100, 500, 5000 or 10000 ppm of carbendazim (equivalent to approximately 0, 5, 25, 250 or 500 mg/kg bw/d, respectively). The parental animals were fed the experimental diet at 21 days of age and mated to produce the F_1 litter at 100 days of age; the numbers of matings, pregnancies, and young were recorded for each litter at birth. The litters were culled to 10 pups on day 4. The number of live pups was again recorded on days 4, 12, and 21, as was pup weight at weaning. The parents were mated again to produce F_{1b} litters, which were maintained on the respective diets for 110 days and then mated to produce the F_{2a} and F_{2b} litters; F_{3a} and F_{3b} litters were produced similarly. Selected tissues and organs from two males and two females in each of five F_{3b} litters from the controls and from the groups fed 5000 and 10000 ppm were examined grossly and histopathologically.

Results: Reproduction indices, including mating, fecundity, fertility, gestation, viability, and lactation, were calculated and compared with control values. Carbendazim had no effect on fertility, gestation, viability, or lactation. The average litter weights at weaning were slightly reduced in all generations at 5000 and 10000 ppm (less than 10%). Histopathological

examination of F_{3b} weanlings did not reveal any effects that were considered to be compound-related. The NOEL was 500 ppm (25 mg/kg bw/d). This study is not suitable for regulatory purposes, due to the lack of maternal observations. Changes in maternal bodyweight, bodyweight gain or food consumption were not recorded, in addition no maternal clinical signs were reported. Histopathology examinations were only completed for the F_{3B} generation and not for either maternal animals, or the F_2 generation.

8. DEVELOPMENTAL STUDIES

The following developmental studies are reproduced from the JMPR report (2005).

Hofmann, H.T. & Peh, J. (1987a) Report on the study to determine the prenatal toxicity of methyl benzimidazole-2-carbamate (MBC) in rats. Unpublished report No. 87/091, Doc. No. A52506 from BASF AG, Ludwigshafen, Germany. Previously submitted to WHO from BASF AG, Ludwigshafen, Germany.

GLP & QA: Statement of compliance with GLP regulations (US FDA, 22nd December 1978); QA statement

Materials and Methods: Carbendazim (purity not indicated) in carboxy methylcellulose was administered by oral gavage to groups of 15-26 pregnant SD rats/group from gestational days (gd) 6-15 at doses of 0, 10, 30, 60, 100, 300, 1000 or 6000 mg/kg bw/d. The dose volume was 1 mL/kg bw. No rationale was given for the dose selection.

Male and female rate had been obtained from Charles River Breeding Laboratories Inc (Portage, Michigan, USA) and were acclimatised for two weeks prior to mating. One male was housed with two females until evidence of mating was detected. The day that mating was confirmed (by examining vaginal smears for the presence of sperm) was designated as gd 0. The bodyweight range for females at the start of the study was 212-247 g. Outside of the mating period, rats were housed individually under standard conditions, with food and water available *ad libitum*.

Observations for mortalities and clinical signs were made twice and once daily, respectively. Dams were weighed on gd 0, 6, 11, 15 and 20. A qualitative analysis of food consumption was made daily. All dams were sacrificed on gd 20 by carbon dioxide asphyxiation and all foetuses removed by caesarean section. The dams were killed on day 20 of gestation, and the reproductive tract was examined for corpora lutea, implantation and resorption sites, and live and dead foetuses. The foetuses were weighed, sexed and examined for external abnormalities. Two thirds of the live foetuses from each litter were selected at random and examined for skeletal alterations, the remaining part of each litter was prepared and examined for soft-tissue alterations.

Results: There were no evident clinical signs of toxicity in dams treated at 10 or 30 mg/kg bw/d. Higher doses resulted in abortion (at 60 and 100 mg/kg bw/d) and signs of toxicity such as tremor and gasping breathing after tactile stimulation, diarrhoea and atactic gait. Mortality was observed at 60 mg/kg bw/d in one dam and in 13 dams at the highest dose. The body-weight gain was similar to that of controls at 10 and 30 mg/kg bw/d and was dose-related, decreasing at 60, 100 and 300 mg/kg bw/d. Animals at 1000 or 3000 mg/kg bw/d lost weight during the dosing period. At necropsy, dams at 300 mg/kg bw/d and above were found to have dark brown colouration of the liver and kidneys, while severe dilatation of the duodenum and

jejunum was observed at 1000 mg/kg bw/d and above. In the vehicle control group, nine foetuses from eight litters had malformations affecting the spine, ribs and head (Table 8).

Table 8: Selected findings from a prenatal developmental study in rats

Findings	Dose (mg/kg bw/d)							
	0	**10**	**30**	**60**	**100**	**300**	**1000**	**3000**
No. of animals	54	26	22	24	22	31	30	29
No. of pregnant animals	51	23	21	23	15	26	25	16
No. of dead animals	0	0	0	1	0	0	0	13
Abortions	0	0	0	2	3	0	0	0
Bodyweight gain (g) days 0-15	49.3	47.1	47.7	39.5**	37.3**	18.7**	-17.3**	-41.3**
Implantations/dam	11	11	9.3	11.5	10	10.9	11.9	9.8
Post-implantation loss (%)	4.9	3.8	6.1	50.7**	85.4**	100**	100**	100**
Live foetuses/dam	10.6	10.6	9.7	5.4	0.3	0	0	0
Mean foetal weight (g)	3.7	3.5	2.9**	2.3**	1.7**	-	-	-
No. of foetuses	309	244	184	116	4	0	0	0
No. of malformed foetuses	9	1	77	104	4	-	-	-
Malformed foetuses (%)	1.7	0.4	42	89	100	-	-	-
Litters affected	8	1	19	18	3	-	-	-

**$p < 0.01$

At 30 mg/kg bw/d, there was a significant decrease in mean foetal weight, and one out of three foetuses was runts. Seventy-seven (42%) foetuses in 19 out of 21 litters had malformations affecting the spine, ribs, head (internal hydrocephalus) and sternum. There was also some increase in variations and delays.

At 60 mg/kg bw/d, two out of 23 animals aborted and 51% of the implantations in the remaining dams were dead. The mean foetal weight was significantly lower than in controls, and virtually all the foetuses were runts. Malformations were seen in 104 (90%) of foetuses from 18 litters. There was also an increase in variations and retardations.

At 100 mg/kg bw/d, 15 pregnant animals produced only four live foetuses in three litters. All foetuses were runts and had malformations of the spine, ribs, limbs, heart, lungs and head. At 300, 1000, and 3000 mg/kg bw/d, all the embryos died in the early and intermediate phases of gestation.

Conclusion: The NOEL for maternal toxicity was 30 mg/kg bw/d based on reduced body-weight gain at 60 mg/kg bw/d and above. The NOEL for developmental toxicity and teratogenicity was 10 mg/kg bw/d based on dose-related reduced foetal weight and increased incidence of malformations at 30 mg/kg bw/d and above.

Hofmann, H.T. & Peh, J. (1987b) Report on the study to determine the prenatal toxicity of methyl benzimidazole-2-carbamate (MBC) in rats. Unpublished report No. 87/092, Doc. No. A52505 from BASF AG, Ludwigshafen, Germany. Previously submitted to WHO from BASF AG, Ludwigshafen, Germany.

GLP & QA: Statement of compliance with GLP regulations (US FDA, 22nd December 1978); QA statement

Materials and Methods: In a follow-up study of prenatal developmental toxicity by the same authors intended to identify a NOEL for malformations, particularly internal hydrocephalus, carbendazim (purity not indicated) was administered at daily doses 10 and 30 mg/kg bw/d by

oral gavage in a 0.5% aqueous suspension of carboxy methylcellulose to groups of 29 or 30 Sprague-Dawley rats on days 6–15 of gestation. The methods were similar to the study performed by the same author *(Hofmann & Peh 1987a).*

Results: No evidence of maternal toxicity was observed. In the control group, four foetuses from four litters had malformations (head, ribs and spine). At 10 mg/kg bw/d, two foetuses from two litters had malformations (head and spine). There was no increase in variations or delays (Table 9).

At 30 mg/kg bw/d, mean foetal weight was significantly decreased, and the number of runts was increased. Eighty-one (23%) foetuses from 22 litters had malformations affecting the head, the spine and the ribs. Internal hydrocephalus was found in 17 (4.8%) foetuses, while 60 or 8 foetuses (16.8% or 2.2%) had cleft thoracic or lumbar vertebrae, respectively. In addition, there was an increase in variations and retardations in the group receiving the highest dose (mainly aplasia and/or displacement of individual sternebrae, dilatation of lateral ventricles in the brain).

Table 9: Selected findings from a prenatal developmental study in rats

Findings	**Dose (mg/kg bw/d)**		
	0	**10**	**30**
No. of animals	20	29	30
Implantations/dam	12	12.4	12.6
Post-implantation loss (%)	4.1	3.6	5.3
Live foetuses	230	346	358
Mean foetal weight (g)	3.47	3.57	3.13**
Runts	2	0	29
Malformation (internal hydrocephalus)	2	1	17
Malformation (cleft thoracic/lumbar vertebrae)	1/0	1/0	60/8
Variation (aplasia of individual sternebrae)	10	12	34
Variation (displacement of individual sternebrae)	1	2	19
Variation (dilatation of lateral ventricles)	1	1	12
Variation of recess of 4th ventricle/Sylvius aqueduct	1	1	5

**$p < 0.01$

Conclusion: The NOEL for maternal toxicity was 30 mg/kg bw/d, the highest dose tested. The NOEL for developmental toxicity and teratogenicity was 10 mg/kg bw/d on the basis of reduced foetal bodyweight and increased incidence of malformations and variations at 30 mg/kg bw/d.

Alvarez, L. (1987) Teratogenicity study of INE-965 (carbendazim) in rats. Unpublished report No MR- 7976-001 HLR 281-87 from E.I. DuPont de Nemours and Co., Haskell Laboratory, Newark, Delaware, USA. Previously submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.

GLP & QA: Statement of compliance with GLP regulations (OECD 414)

Materials and Methods: In a study of prenatal developmental toxicity, groups of 25 Crl:CD BR rats were given carbendazim (purity, 98.8%) at a dose of 0, 5, 10, 20 or 90 mg/kg bw/d by gavage in a 0.5% aqueous suspension of carboxy methylcellulose on days 7–16 of gestation. The dams were killed on day 22 of gestation, and the reproductive tract was examined for corpora lutea, implantation and resorption sites and live and dead foetuses. The foetuses were

weighed, sexed and examined for external abnormalities. For each litter, the maximum stunted weight was calculated by subtracting the lightest weight from the total weight, dividing by the remaining number of foetuses and multiplying by 0.666. A foetus weighing the same or less than the maximum stunted weight was considered to be stunted; its weight was omitted when the mean litter weight was calculated. The first live foetus and thereafter every other foetus in each litter was decapitated and examined for visceral alternations and the sex verified. The heads were fixed in Bouin' fluid and examined. All stunted and externally malformed foetuses were also examined for visceral alterations; a decision to do a head examination was made on an individual basis. All remaining foetuses were stained skeletally with alizarin red S and examined for skeletal alterations.

Results: Maternal toxicity was seen only at 90 mg/kg bw/d in the form of reduced bodyweight gains. In addition, mean liver weights were increased at this dose.

Table 10: Selected findings from a prenatal developmental study in rats

	Dose (mg/kg bw/d)				
	0	**5**	**10**	**20**	**90**
	Maternal findings				
Bodyweight gains (g) days 7-17	53.3	55.9	52.9	55.5	48.7*
Liver weights (g)	15.5	15.8	16.6	16.8	17.1*
Pregnancy	24	23	24	22	19*
Resorption/dam	13.3	13.7	12.6	13.5	3.5**
Live foetuses/dam	13	13.5	11.7	13.1	9.9*
	Foetal findings				
Mean foetal weight (g) F/M	5.2/5.4	5.2/5.5	5.0/5.4	4.9*/5.1*	3.6**/3.9**
External examination (foetus/litter)	312/24	10/24	281/24	288/22	149/15
Malformations (foetus/litter)	1/1	0	0	0	43/8**
Eye bulge –-absent	0	0	0	0	12/6**
Head - domed	0	0	0	0	9/4**
- exencephaly	0	0	0	0	17/2**
Paw - clubbed	0	0	0	0	11/6**
External examination (foetus/litter)	312/24	10/24	281/24	288/22	149/15
Malformations (foetus/litter)	2/2	0	1/1	1/1	41/14**
Brain - hydrocephalus	0	0	0	0	24/10**
- lateral ventricle distended	0	0	0	0	4/4**
Eye - anophthalmia	0	0	0	0	21/10**
Skeletal examination (foetus/litter)	312/24	10/24	281/24	288/22	149/15
Malformations (foetus/litter)	2/2	0	0	2/2	80/12**
Vertebra - fused	0	0	0	0	77/11**
- hemivertebra	0	0	0	0	23/10**
Rib - fused	0	0	0	0	40/10**
- none	0	0	0	1/1	4/2*
Sternum - fused sternebrae	0	0	0	1/1	12/6
- scrambled sternebrae	0	0	0	1/1	1/1
Scapula - malformed	0	0	0	0	28/6**

*p < 0.05; **p < 0.01

At 90 mg/kg bw/d, the low number of dams delivering pups (15 versus 24 for the control group) was attributed to the lower pregnancy rate (19 of 25 dams). There was one death (by mechanical dosing trauma) and three dams had total resorptions. The increased mortality *in utero* at this dose was mainly caused by an increased incidence of early resorptions. Associated with these changes was a significant decrease in litter size (live foetuses per dam), with only the reduction in females per litter being statistically significant. No effect on survival was seen at other doses (Table 10).

At 20 and 90 mg/kg bw/d, embryo- and foetal toxicity was evident as a significant reduction in mean foetal weight. A significant increase in the incidence of malformations was seen in the 90 mg/kg bw/d. These malformations included a variety of conditions mainly in the head (exencephaly, domed head, hydrocephaly), eyes (anophthalmia), paws (clubbed) and skeletal malformations (fused vertebrae, ribs and sternum; hemivertebrae; rib hypoplasia; malformed scapula).

Conclusion: The NOEL for maternal toxicity was 20 mg/kg bw/d on the basis of decreased bodyweight gain and increased liver weight at 90 mg/kg bw/d. The NOEL for developmental toxicity was 10 mg/kg bw/d based decreased foetal bodyweight at 20 mg/kg bw/d and above.

Culik, R., Sherman, H. & Zapp, J.A. (1970) Teratogenic study in rats with 2-benzimidazole-carbamic acid, methyl ester (INE-965). Report No. HLR 466-70 from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.

GLP & QA: not stated

Materials and Methods: In a study of prenatal developmental toxicity, groups of 27–28 pregnant ChR-CD rats were fed diets containing carbendazim as a formulation (carbendazim, 53%) at a concentration of 0, 100, 500, 2500, 5000, 7500, or 10000 ppm (equivalent to 0, 8.9, 46, 218, 432, 626 and 747 mg/kg bw/d from day 6 to day 15 of gestation). After day 15, rats received control diet until sacrifice on day 20, when litters were delivered by caesarean section. The numbers and location of live and dead foetuses and resorption sites, bodyweights, crown–rump length, sex, and visible abnormalities were determined. Two thirds of the foetuses were examined for skeletal defects, and the remainder were examined for visceral and soft-tissue anomalies.

Results: There were no deaths or treatment-related signs of toxicity during the study. Bodyweight and body-weight gain were not adversely affected by treatment. Reproductive parameters (number of implantation and resorption sites, number of females with complete or partial resorption, number of live foetuses and dead foetuses) were not affected by treatment. Foetal bodyweights and the incidences of gross external, skeletal, and soft tissue malformations and variations were similar in all groups.

Conclusion: The NOEL for maternal and developmental toxicity was 10 000 ppm (equivalent to 747 mg/kg bw/d), the highest dose tested.

Christian, N.S et al (1985) Developmental toxicity study of carbendazim administered via gavage to New Zealand white rabbits. Unpublished report, study No. 104-008, from Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Previously submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.

GLP & QA: Statement of compliance with GLP regulations (OECD 414)

Materials and Methods: In a study of prenatal developmental toxicity, groups of 20 artificially inseminated New Zealand White [Hra:(NZW)SPF] rabbits were given carbendazim (purity, 98.7%) at a dose of 0, 10, 20, or 125 mg/kg bw/d by gavage in aqueous 0.5% carboxy

methylcellulose on days 7–19 of presumed gestation. The dams were killed on day 29 of gestation, and the reproductive tract was examined for corpora lutea, implantation and resorption sites and live and dead foetuses. The foetuses were weighed, sexed and examined for external abnormalities and for visceral alterations. The brain was free-hand transverse-sectioned (a single cut at the level of the anterior fontanelle) and examined. The bodies of all foetuses obtained after a minimum of 27 days of gestation were processed, stained with alizarin red S and evaluated for skeletal alterations.

Results: Two rabbits at 125 mg/kg bw/d died, one on day 22 and the other on day 25 of gestation. As this incidence is slightly in excess of the incidence in historical controls, the effect is considered to be treatment-related. Maternal weight gains were reduced at 125 mg/kg bw/d during the dosage period (13%).

The incidence of pregnancy was similar at all doses (Table 11). A slight decrease in the number of corpora lutea and increased resorption were observed at 125 mg/kg bw/d while decreased implantation, increased resorption and decreased live litter size were seen at 20 and 125 mg/kg bw/d. The average percentage of malformed foetuses per litter was significantly increased at 125 mg/kg bw/d. Treatment-related malformations at 125 mg/kg bw/d consisted of malformed cervical vertebrae and interrelated malformations of the ribs and proximate thoracic vertebrae.

Conclusions: The NOEL for maternal toxicity was 20 mg/kg bw/d based on a slight increase in abortions and decreased bodyweight gain at 125 mg/kg bw/d. The NOEL for developmental toxicity was 10 mg/kg bw/d based decreased implantation, increased resorption and decreased live litter size at 20 mg/kg bw/d.

Table 11: Selected findings from a prenatal developmental study in rabbits

Findings	**Dose (mg/kg bw/d)**			
	0	**10**	**20**	**125**
Pregnancy	16/20	17/20	17/20	18/20
Abortion	0	0	0	2
Mean corpora lutea	10.7	11.3	10.5	8.9*
Mean implantations	7.7	7.4	6*	5.9*
Mean resorptions	0.2	1	0.6	2.9*
Mean foetal weight (g)	43.32	44.10	42.15	40.83*
Litters with total absorptions	0	0	1	7**
Live foetuses (total)	105	103	91	49*
Mean live foetuses/litter	7.5	6.4	5.7*	5.4*
Foetuses with alterations/malformations (%)	19/10	18/10	18/9	54/53**

*p < 0.05; **p < 0.01

9. GENOTOXICITY

Tables 12 and 13 summarises submitted and published findings of *in vitro* and *in vivo* genotoxicity studies for carbendazim. *In vitro* and *in vivo* mechanistic studies are described in more details.

Table 12: Summary of *In Vitro* Genotoxicity Studies

Assay/endpoints	Species, Strains	Concentration, Purity	Metabolic Activation	Result	Reference
Gene Mutation					
Reverse mutation in bacteria	*S. typhimurium TA97a, TA98, TA100, TA1535, TA1538*	0.001–5.0 μg/plate, 99.5% purity	+,-	-	Ranzani *et al* (2001a)
Reverse mutation in bacteria	*S. typhimurium TA97a, TA98, TA100, TA1535, TA1538*	0.001–5.0 μg/plate, 99.5% purity	+,-	-	Ranzani *et al* (2001b)
Reverse mutation in bacteria	*S. typhimurium TA97, TA98, TA100, TA102*	2.5–2500 μg/plate, 98% purity	+,-	-	Yang *et al* (1997)
Reverse mutation in bacteria	*S. typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2uvrA*	0.4–250 μg/plate, 99% purity	+,-	-	Ming *et al* (2001)
TK+/- gene mutation assays	Mouse Lymphoma L5178Y cells	0–100 μM, 99.5% purity	+,-	-	Adams *et al* (1996)
Polyploidy induction &chromosomal aberrations	Chinese hamster lung cells	0.78-100 μg/mL, 99.5%	+,-	+ for polyploidy (all doses) - for clastogenic activity	Kitching *et al* (1996a)
Polyploidy induction &chromosomal aberrations	Human lymphocytes	0.78-100 μg/mL, 99.5%	+,-	+ for polyploidy (all doses) - for clastogenic activity	Kitching *et al* (1996b)
Aneuploidy induction	Human lymphocytes	0-5 μg/mL, 99.7%	+,-	+ ≥ 0.6 μg/mL (NOEL 0.5 μg/mL)	Marshall *et al* (1996)#
Aneuploidy induction	Human lymphocytes	0-2 μg/mL, 99.7%	+,-	+ ≥ 0.6 μg/mL (NOEL 0.5 μg/mL)	Bentley *et al* (2000)#
Aneuploidy induction	Human lymphocytes	0-2 μg/mL, 99.5%	+,-	+ ≥ 0.2 μg/mL	Elhajouji *et al* (1995, 1997)#
Disruption of meiotic cell cycle	CF-1 mouse oocytes	0-6 μg/mL, 99.7%	+,-	+ ≥ 0.2 μg/mL	Can (1997)#

Results (-, negative; +, positive) are expressed relative to the presence (+) or absence (-) of metabolic activation. NR = Not reported
Results are from JMPR evaluations and were not independently evaluated by OCSEH.

Table 13: Summary of *In Vivo* Genotoxicity Studies.

End Points/tests	Species, Strains	Concentration	Result	Reference
Chromosomal Effect Assays				
Micronucleus formation/Bone marrow micronucleus test	NMRI mouse bone marrow	2 doses (24 hour-apart) gavage dose of 0, 50, 500, 5000 mg/kg bw	+ ≥ 500 mg/kg bw	Mayer (1980a)#
	NMRI mouse bone marrow	2 doses (24 hour-apart) gavage dose of 0, 50, 500, 5000 mg/kg bw	+ ≥ 500 mg/kg bw	Mayer (1980b)#
	NMRI mouse bone marrow	1 dose gavage dose of 50 or 200 mg/kg bw	-	Muller (1990)#
	B6D2F1/Cr-1BR	Single gavage dose of 66, 1646, 3293 mg/kg bw	+ ≥ 1646 mg/kg bw	Bentley (1990)#; Sarrief *et al* (1994)#
	Wistar rat bone marrow	Single gavage dose of 150 mg/kg bw	+	Ashby (2001)#
	Swiss mouse colon epithelial cells	Single gavage dose of 500 or 1000 mg/kg bw	+ ≥ 500 mg/kg bw	Vanhauwaert *et al* (2001)#
	Sprague-Dawley rat spermatids	Single gavage dose of 50, 100 or 400 mg/kg bw	+ ≥ 50 mg/kg bw	Matsuo *et al* (1999)#
	Kunming mice	Single gavage dose of 400, 800 or 1600 mg/kg bw	-	Yu (1997)
	Swiss albino mice	Single gavage dose of 1000, 2000 or 3000 mg/kg bw	-	Yu (1997)
Aneuploidy induction	Syrian hamster oocytes	Single gavage dose of 1000 mg/kg bw	+	Costa *et al* (2001)#
	Wistar rat sperm	Single gavage dose of 50, 150, 450 or 800 mg/kg bw	+ ≥ 800 mg/kg bw	De Stopplaar *et al* (1999)#
Gene Mutation				
Dominant lethal test	NMRI mouse	Single dose of 1280 mg/kg bw intraperitoneally	-	Hofmann & Peh (1973)#

Results (-, negative; +, positive)
Results are from JMPR evaluations and were not independently evaluated by OCSEH.

9.1 *IN VITRO* GENOTOXICITY STUDIES

Bentley, K.S. et al (2000) Evaluation of thresholds for benomyl- and carbendazim-induced aneuploidy in cultured human lymphocytes using fluorescence in situ hybridization. Mutat Res 464: 41–51.

Materials and methods: The *in vitro* human lymphocyte micronucleus test in combination with chromosome-specific centromeric probes (FISH) was used to assess the degree of aneuploidy *in vitro* induced by either benomyl (95% purity) or carbendazim (97% purity). Human lymphocytes were incubated with either benomyl or carbendazim without metabolic activation in the range 0 - 500 μM. In aqueous media, the half-life of benomyl has been reported to be approximately 2 h, thus it was assumed that under the study conditions, that benomyl would dissociate to carbendazim and n-butyl-isocyanate.

Results: The authors reported that the induction of aneuploidy (via mitotic spindle inhibition) by benomyl and carbendazim exhibited a dose-response relationship, which included a threshold level. Both compounds were found to induce aneuploidy, exhibiting similar threshold levels of 3.2-4.3 and 3.8-4.1 μM for carbendazim and benomyl respectively.

Marshall, R. (1996) Carbendazim: Induction of aneuploidy in cultured human peripheral blood lymphocytes. HLO 506-96 from Corning Hazleton, Harrogate, England. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.

In order to demonstrate the existence of a threshold level for the induction of aneuploidy by carbendazim (purity, 99.7%), a modified test for micronucleus formation *in vitro* was used coupled with FISH to determine the distribution of chromosomes between daughter nuclei in cytokinesis-blocked binucleated human lymphocytes. Slide preparations were hybridized with centromeric DNA probes specific for six different human chromosomes: 1 and 8 (trisomy's associated with a number of tumour types), 11 (associated with Wilm's tumour), 17 (associated with *p53* gene and breast cancer predisposition), and X and 18 (frequently associated with live aneuploidies). One thousand cells on two replicate slides were evaluated; abnormalities in chromosome distribution were classified as chromosome loss, chromosome gain, non-disjunction, or polyploidy. The presence of micronuclei carrying a centromeric signal was also recorded. The threshold was defined as the lowest concentration that produced a statistically significant increase in aneuploidy. At low concentrations, the frequencies of non-disjunction, chromosome loss, and chromosome gain were similar to control levels until specific points within the dosing range when concentration-dependent increases occurred for each end-point. As a result, one daughter cell contains two copies of one chromosome, while the other daughter cell lacks that chromosome occurred at higher frequencies and generally at lower concentrations than for chromosome loss and chromosome gain and was considered to be the most sensitive aneugenic event for determining a threshold. For each end-point, the shapes of the dose–response curves for the six chromosomes were nearly identical. Threshold concentrations for non-disjunction caused by carbendazim were 600 ng/ml for chromosomes 17 and X, 700 ng/ml for chromosome 1, and 800 ng/ml for chromosomes 8, 11, and 18.

Elhajouji A et al. (1997) Indication for thresholds of chromosome nondisjunction versus chromosome lagging induced by spindle inhibitors in vitro in human lymphocytes. Mutagenesis 12: 133–140.

and

Elhajouji et al. (1995) Indications for a threshold of chemically induced aneuploidy in vitro in human lymphocytes. Environ. Mol. Mutagen., 26: 292–304.

A threshold mechanism of action was also reported for carbendazim (purity not reported) and three other well-known mitotic spindle poisons (cholchicine, mebendazole, and nocodazole) using a similar experimental test system *in vitro* with cultured human lymphocytes, as in Marshall (1996a). Chromosome loss was analysed by identifying micronuclei with centromeres using a fluorescent human pancentromeric DNA probe. Non-disjunction was evaluated in cytokinesis-blocked binucleated lymphocytes in combination with centromeric probes for chromosomes 1 and 17. For both end-points, similarly shaped dose–response curves were obtained for the four mitotic spindle inhibitors. In each case, the first concentration at which a statistically significant increase occurred was greater for chromosome loss than for non-disjunction. For carbendazim, the threshold concentration for non-disjunction was 1.05 μmol/l, corresponding to about 0.2 μg/ml.

Can, A. & Albertini, D.F. (1997) Stage specific effects of carbendazim (MBC) on meiotic cell cycle progression in mouse oocytes. Mol Reprod Dev 46: 351–362.

The meiotic maturation *in vitro* of cultured mouse (CF-1, Harlan Sprague-Dawley) oocytes was evaluated after exposure to carbendazim (purity, 99.7%) at a concentration of 0, 3, 10, or 30 μmol/l (corresponding to 0, 600, 2000, and 6000 ng/ml). Exposures were for a period of 6–8 h during initial stages of in-vitro maturation and meiotic metaphase I, during a 8–9 h interval between metaphase I and metaphase II, or 14–16 h during the entire period of maturation. Meiotic maturation was assessed in respect to chromosome organization, meiotic spindle microtubules, and cortical actin using fluorescent labelling for each of the structures. Exposure to carbendazim resulted in a dose-dependent inhibition of cell cycle progression at meiotic metaphase I, but did not interfere with progress to metaphase II except at the highest concentration. A loss in nonacetylated microtubules and a decrease in spindle size were noted at 3 and 10 μmol/l. At a concentration of 30 μmol/l, spindle assembly was prevented when carbendazim was added at the beginning of meiotic maturation or spindle pole disruption and fragmentation were caused when carbendazim was added to preformed spindles. Dispersed chromosomes were retained in the metaphase-plate location. Polar body extrusion was also impaired, and abnormal polar bodies were observed in most treated oocytes. The results of this study suggested that carbendazim disrupts cell cycle progress in oocytes by altering meiotic spindle microtubule stability and spindle pole integrity.

9.2 *IN VIVO* GENOTOXICITY STUDIES

Ashby, J. & Tinwell, H. (2001) Continuing ability of the rodent bone marrow micronucleus assay to act as a predictor of possible germ cell mutagenicity of chemicals. Mutat Res 478: 211–213.

Wistar-derived rats received carbendazim (source and purity not reported) as either one or two oral doses at 150 mg/kg bw suspended in corn oil; concurrent controls were given corn oil alone. All animals were terminated 24 h after the last dose, and 2000 polychromatic erythrocytes were evaluated from bone-marrow smears. Carbendazim induced a statistically significant and reproducible increase in the incidence of micronuclei, confirming the induction of aneuploidy in the somatic cells of the rat bone marrow.

Vanhauwaert, A et al (2001) The in vivo gut micronucleus test detects clastogens and aneugens given by gavage. Mutagenesis 16:39–50.

SPF albino Swiss mice were given carbendazim (purity, 97%) as a single oral dose at either 500 or 1000 mg/kg bw in Methocel-Tween. Animals were terminated at either 24 or 48 h after exposure. One thousand epithelial cells within the intestinal crypts were evaluated for the presence of micronuclei. Carbendazim appeared to induce a small but statistically significant increase in the frequency of micronuclei at both doses and sampling intervals, but a dose–response relationship was not observed. Although the authors stated that an increase of micronuclei did not occur in the accompanying evaluation of bone marrow, the data presented show a fivefold and threefold increase (not statistically significant) in micro-nucleated polychromatic erythrocytes at the 24 h sampling interval after carbendazim doses of 500 and 1000 mg/kg bw, respectively.

Jeffay, S.C et al (1996) Acute exposure of female hamsters to carbendazim (MBC) during meiosis results in aneuploid oocytes with subsequent arrest of embryonic cleavage and implantation. Reprod Toxicol 10: 183–189.

In a study conducted to assess the ability of carbendazim to induce aneuploidy in oocytes, female Syrian hamsters were given carbendazim (purity, 95%) as a single oral dose at 1000 mg/kg bw in corn oil on the afternoon of proestrus coinciding with meiotic maturation of the oocytes. In unfertilized oocytes, the treatment resulted in an apparent increase in aneuploidy frequency (37.2% compared with 13.5% in the control group) as indicated by cytogenetic analysis of the chromosome number. There was no evidence for any carbendazim-induced structural abnormalities. In animals that were allowed to mate after dosing, fertilization rate and number of oocytes recovered was not impaired. However, pre-implantation embryonic development was affected as evidenced by an increase in the proportion of embryos that failed to reach the expected stages of development (e.g. eight-cell, morula, or blastocyst stages). In addition, the mean number of implantation sites was lowered.

Matsuo, et al. (1999) The fungicide carbendazim induces meiotic micronuclei in the spermatids of the rat testis. J Vet Med Sci 61: 573–576.

In a study conducted to assess the ability of carbendazim to induce micronucleus formation in round (step I, immature) spermatids, groups of six male Sprague-Dawley rats were given

carbendazim (source and purity not reported) as single oral doses at 0, 50, 100 or 400 mg/kg bw in corn oil. The animals were sacrificed 24 h after dosing, and following staining for DNA, 1500 spermatids per individual rat, were evaluated for the formation of micronuclei. A statistically significant increase was observed only in the group at receiving the intermediate dose; frequencies of micronuclei were nearly identical at 50 and 400 mg/kg bw, showing the absence of a dose–response relationship. The authors suspected that testicular damage at the highest dose may have been responsible for reduced frequencies of micro-nucleated spermatids. Immunocytochemistry of spermatids from the group receiving a dose of 100 mg/kg bw indicated that as much as 68% of the micronuclei contained kinetochores compared with 30% in the control group. The authors concluded that carbendazim-induced micronuclei in rat spermatids were caused by aneuploidy, rather than possible clastogenic activity of the test compound.

de Stoppelaar, J.M et al (1999) Increased frequencies of diploid sperm detected by multicolour FISH after treatment of rats with carbendazim without micronucleus induction in peripheral blood erythrocytes. Mutagenesis 14: 621–631.

In a study conducted to assess the ability of carbendazim to induce numerical chromosome aberrations in sperm and micronuclei in peripheral blood erythrocytes, groups of three to five Wistar rats received carbendazim (purity not reported) as single oral doses at 0, 50, 150, 450, or 800 mg/kg bw in corn oil. Dual-colour FISH was performed on epididymal sperm obtained 31 days after treatment using probes for chromosomes 4 and Y. At least 10000 sperm per animal were evaluated. Further categorization of hyperhaploid sperm (44Y, 4YY, 44) by nuclear size was conducted to evaluate which sperm were actually diploid. The authors reported that increases in hyperploid sperm were observed at doses of 150 mg/kg bw and greater and that, based on evaluation of nuclear size, the majority were diploid sperm. A subsequent experiment was conducted using tri-colour FISH using probes for two somatic chromosomes 4 and 19 and for chromosome Y. As noted by the authors, the three-chromosome analysis was considered to be a more accurate method for assessing aneuploidy and diploidy in sperm. In this second experiment, 11 doses of carbendazim were used ranging from 2.5 to 800 mg/kg bw; each group contained three animals. In addition, 2000 peripheral blood erythrocytes per animal were evaluated for the presence of micronuclei in blood samples taken 48 h after treatment. The results showed a preferential induction of diploid sperm by carbendazim, but only at the highest dose tested, 800 mg/kg bw, and the frequency was lower than that reported in the first experiment where nuclear size had been considered for classification of aneuploid and diploid sperm. No increases in micronuclei were observed in peripheral erythrocytes at any carbendazim dose. On the basis of this study, the authors suggested that diploidy could be induced in sperm at a lower dose than could micronucleus formation in peripheral blood erythrocytes. However, it should be noted that analysis of peripheral blood samples for micronuclei in this study was likely suboptimal at the selected sampling time as micro-nucleated erythrocytes may have been efficiently removed by the spleen in rats.

10. TESTICULAR TOXICITY

Evenson, D.P., Janca, F.C. & Jost, L.K. (1987) Effects of the fungicide methyl-benzimidazol-2-yl carbamate (MBC) on mouse germ cells as determined by flow cytometry. J. Toxicol. Environ. Health, 20, 387-399.

In this study, dual-parameter (DNA, RNA) flow cytometry (FCM) measurements were made on testicular and epididymal sperm cells isolated from mice exposed by oral gavage to 0, 250, 500, or 1000 mg/kg bw/d carbendazim for 5 days. Effects of exposure to carbendazim were measured at 7, 24, and 39 days post-treatment. Carbendazim had no effect on bodyweights, however, testis weights and sperm parameters were altered at 1000 mg/kg bw/d. Testis weights were reduced by about 25% at 7 and 24 days after exposure but recovery was observed by 39 days after treatment. FCM measurements of testicular cells showed relative percentages of certain testicular populations (round, elongating, and elongated spermatids) were different from the control pattern 7 and 24 d after treatment. The mean percent of cauda epididymal sperm head morphology abnormalities and the susceptibility of the nuclear DNA to denaturation were both elevated at 7, 24, and 39 d after exposure to 1000 mg/kg bw/d. These data demonstrate that spermatogenesis is sensitive to high-dose carbendazim exposure resulting in an altered ratio of testicular cell types present, abnormal sperm head morphology, and an altered sperm chromatin structure.

Nakai, M et al (1992) Acute and long-term effects of a single dose of the fungicide carbendazim (methyl 2-benzimidazole carbamate) on the male reproductive system in the rat. J. Androl., 13: 507–518.

Materials and methods: In this report, groups of 20 male rats aged 97–105 days were given carbendazim as a single oral dose at 0, 50, 100, 200, 400 or 800 mg/kg bw (purity not indicated) and were killed 2 days (8 animals/dose) or 70 days (12 animals/dose) after treatment. On day 2, at 50 mg/kg bw, round spermatids were sloughed (prematurely released) from stage I and II epithelium and elongated spermatids were sloughed from stage VII epithelium. The percentage of seminiferous tubules exhibiting epithelial sloughing was only marginally increased (less than 10%) and not statistically significantly different from the controls. At 100 mg/kg bw, the disappearance of germ cells was more severe and sloughing of elongated spermatids extended into stages XII through XIV. In animals treated at 100 mg/kg bw or more, a statistically significant and dose-dependent increase in testicular weight was seen that was accompanied by significant increases in mean seminiferous tubular diameter at 400 and 800 mg/kg bw. In addition, at 100 mg/kg bw and above, there was a dose-dependent increased incidence of occlusions in the efferent ductules of the testes. The rete testis was swollen with sloughed germ cells, indicating that ductal blockage had occurred further down the tract; 50% or more of the efferent ductules were occluded. The occlusions were characterized as compacted luminal contents, spermatic granulomas, mineralisation, and obliteration of the original lumen by fibrotic connective tissue. At 200 mg/kg bw and above, missing germ cells extended into at all stages except stages IX–XI, while 400 and 800 mg/kg bw, some seminiferous epithelia were damaged so severely that it was difficult to identify the stage.

Results: On day 70, mean seminiferous tubule diameter was statistically significantly decreased at all doses in a dose-dependent relationship. Histologically, these decreases were associated with a dose-dependent increase in seminiferous tubular atrophy (statistically

significant at 100 mg/kg bw). No atrophic tubules were seen in the control rats, however, atrophy of a few seminiferous tubules in one testicle was noted at 50 mg/kg bw. The atrophied tubules contained primarily Sertoli cells and occasional spermatogonia and were surrounded by a thickened basement membrane. Pathological alterations were also noted in the efferent ductules of the treated animals, 50% or more of the ducts being occluded in rats dosed at 100 mg/kg bw. Minimal effects were seen at 50 mg/kg bw; slight abnormal growth of the efferent ductules was seen in only one specimen. The occlusions were characterized as compacted luminal contents, spermatic granulomas, mineralisation and obliterations of the original lumen by fibrotic connective tissue. In addition, mean testis weight showed a dose-dependent decrease that was statistically significant at doses of 100 mg/kg bw and above.

Conclusions: Effects at 50 mg/kg bw were considered toxicologically significant due to the strong dose-relationship of effects. No NOEL was established because of testicular toxicity seen at the lowest dose (premature release of immature germ cells 2 days post exposure, atrophy of seminiferous tubules, decreased seminiferous tubule diameter and abnormal growth of efferent ductules). These effects persisted for at least 70 days.

Lim J & Miller MG (1997) The Role of the Benomyl Metabolite Carbendazim in Benomyl-Induced Testicular Toxicity in Rats. Department of Environmental Toxicology. University of California, Davis. Toxicol Appl Pharmacol 142: 401-410

The role of benomyl (>95% purity) and carbendazim (>95% purity) in compound-related testicular toxicity was investigated. After ip administration (859 μmol/kg bw) of equimolar concentrations of benomyl and carbendazim to rats, carbendazim caused sloughing of the seminiferous epithelium after 1 h, whereas no significant effect was observed for benomyl after 1 h. Using an area under curve (AUC) to express testicular concentration levels over 2 h, a good linear correlation was observed between percent sloughing and AUC for carbendazim. A similar correlation was observed for benomyl, although it was less potent. The sloughing observed after benomyl treatment was best correlated to the amount of carbendazim (derived from benomyl metabolism) in the testis.

The ability of purified testicular tubulin proteins to assemble into microtubules *in vitro* could be monitored spectrophotometrically. This microtubule assembly could be inhibited by carbendazim and benomyl with IC_{50}'s of 5 μM and 75 μM respectively. These results suggest that carbendazim, and not benomyl itself, is the mediator of testicular toxicity and an inhibitor of testicular microtubule assembly.

Nakai M & Hess RA (1997) Effects of carbendazim (methyl 2-benzimidazole carbamate; MBC) on meiotic spermatocytes and subsequent spermiogenesis in the rat testis. Anat Rec 247:379-387.

This study determines the direct effects on dividing germ cells and the subsequent effects on spermiogenesis. Carbendazim was administered orally to male rats (100 mg/kg), and their testes were processed for histological evaluation at various post-treatment intervals up to day 20. The sloughing of elongate spermatids was observed as reported previously. In addition to this Sertoli cell lesion, necrosis of dividing spermatocytes in stage XIV was observed at 8 hours post-treatment. At day 1.5, empty spaces of missing step 1 spermatids were seen in stage I. At days 4.5 and 7.5, normal round spermatids were missing, but large round spermatids (megaspermatids) and binucleate spermatids were common. The megaspermatid nucleus was approximately 33% larger in diameter than normal round spermatids. At day

10.5, megasteps 10-12 spermatids, binucleate spermatids, and three to four different steps of spermatids coexisting in the same tubule section were present in stages X-XII. In addition, abnormally shaped elongating spermatids were observed having distorted heads and nuclear invagination containing microtubules. At day 20, empty spaces of missing diplotene spermatocytes were seen in stage XIII. These observations show that carbendazim has rapid direct effects on meiotic spermatocytes and latent effects on spermatids, leading to morphological abnormalities and failure of spermiogenesis. These effects are found independent of occlusions in the efferent ductules.

Winder BS et al (2001) The role of GTP Binding and microtubule-associated proteins in the inhibition of microtubule assembly by carbendazim. Toxicol Scien 59:138-146.

This study investigated the mechanism underlying the toxicity of carbendazim. Tubulin and microtubule-associated proteins (MAPs) were isolated from rat testis and brain. The effects of carbendazim on microtubular assembly were compared with the known microtubule (MT) disruptors, colchicine and nocodazole. Carbendazim (100 μM) had no effect on the assembly of MTs from MAP-containing tubulin isolated with one cycle of glycerol-dependent assembly and disassembly while colchicine (40 μM) and nocodazole (12.5 μM) strongly inhibited the assembly reaction. Similarly, formation of MTs from tubulin prepared with two cycles of glycerol-dependent assembly was strongly inhibited by colchicine and nocodazole but only weakly by carbendazim. All three compounds inhibited the assembly of MTs from MAP-free tubulin isolated with glutamate. However, the inhibition by carbendazim was reversed by the inclusion of high-molecular-weight MAPs and not by unrelated protein (bovine serum albumin, BSA). Addition of nocodazole to assembled MTs caused immediate depolymerization, whereas carbendazim did not directly cause depolymerization. However carbendazim was an effective inhibitor of the polymerization of depolymerized tubulin. In competitive binding assays, carbendazim was found to inhibit the binding of guanosine triphosphate (GTP) to tubulin. The data suggest that carbendazim interferes with initial events of MT polymerization, specifically GTP binding, and that MAPs moderate this effect.

11. NEUROTOXICITY

Goldenthal, E.I. (1978) Neurotoxicity in hens. Unpublished report from International Research and Development Corporation, Mattawan, Michigan, USA. Submitted to WHO by E.I. DuPont de Nemours and Co., Inc.

Groups of 10 white Leghorn hens received a single dose of carbendazim at 500, 2500, or 5000 mg/kg bw to test for delayed neurotoxic potential. Controls received the vehicle, corn oil, and the neurotoxin tri-*ortho*-tolyl phosphate. The hens were observed daily for mortality and clinical neurotoxicity for four weeks. Neurotoxic signs consisting of leg weakness, ataxia and/or 'goose-stepping' gait were observed in hens treated with tri- *ortho*-tolyl phosphate. Less severe, reversible signs, consisting of slight leg weakness and ataxia, were observed in hens treated with carbendazim at 5000 mg/kg bw, but no neurotoxic signs were observed in those treated at lower doses. Microscopic examination indicated no axonal degeneration or demyelination in carbendazim-treated animals.

12. HUMAN STUDIES

The following studies are reproduced from the OCSEH benomyl review (2004).

Meuling WJA et al (2000) Dermal absorption of Benlate WP50 in human volunteers. TNO Nutrition and Food Research Institute, Department of Target Organ Toxicity, Zeist, The Netherlands Unpublished Report No. V2662 Project No: 40955 Dated 25 May 2000

and

Meuling WJA (2001) Summary report on the re-analysis of carbendazim (MBC) in plasma samples. TNO Nutrition and Food Research Institute, Department of Target Organ Toxicity, Zeist, The Netherlands Unpublished Report No. V3596 Project No: 010.41215 Dated 5 March 2001

The study was performed according to the ICH Guidelines for Good Clinical Practice at the request of Ferraro & Associates PA of Miami, Florida USA, the legal representatives of a plaintiff in litigation against DuPont. The analytical phase of the study was quality-assured and carried out in accordance with OECD GLP guidelines. The objective was to determine the absorption of benomyl and/or its metabolites in humans after dermal application of Benlate WP50 to variable areas. The urine and blood sampling protocols used in this study were based on experience gained in a previous experiment (Meuling *et al* 1993) in which carbendazim was administered orally, intravenously and applied to the forearm of human volunteers.

Methods

The subject group consisted of 8 healthy volunteers (5 male and 3 female), 22 – 26 yr old. The female subjects were non-pregnant and had a mean bodyweight of 68 kg (range 60 – 83) and body mass index of 23 kg/m^2 (range 21 – 27), while the males had a mean bodyweight of 84 kg (range 78 – 90) and body mass index of 25 kg/m^2 (range 23 – 27). The overall mean bodyweight was 78 kg and body mass index was 25 kg/m^2.

Benlate WP50 (TNO-PBS No. 990345) was purchased commercially and mixed with water at a concentration of 60 mg/mL. A 0.5 mL volume of the suspension was applied to both upper thighs of each subject, for a total dose of 60 mg formulation (30 mg benomyl, or approximately 0.4 mg/kg bw). According to the study protocol, subjects were to have been exposed twice according to a balanced, partially randomised treatment design, in which the dose was held constant but the combined application areas were varied between 300, 600 or 900 cm^2. The respective dermal loadings of benomyl would therefore have been 0.1, 0.05 or 0.033 mg/cm^2. However, technical problems occurred during the initial exposure of 4 subjects, causing the procedure to be repeated after the second scheduled exposure. It is unclear whether the test compound was actually applied before the initial exposure ceased. If so, subjects 1-4 would have been treated a total of 3 times.

The exposure period was 4 h, during which the subjects remained within a controlled environment (temperature 25°C, relative humidity 50%). The remaining test compound was removed by washing at the end of the exposure period and the subjects remained within the laboratory for a further 6 h before returning home. There was a washout period of 5 - 7 d between exposures.

Blood samples were withdrawn through an indwelling cannula and collected prior to treatment and 30, 45, 60, 75, 90, 120, 150, 180, 240 and 480 min after the commencement of treatment. The concentrations of carbendazim and 5-HBC (all subjects) and 3-butyl-1,3,5-triazino(1,2-benzimidazole-2,4(1H,3H)-dione (STB, 1 subject) in plasma were analysed by HPLC. The LOD and LOQ for carbendazim were 3.4 and 8.2 μg/L (ppb), respectively and the corresponding values for STB were 2.4 and 5.7 μg/L (ppb). Due to replacement of the HPLC column, the LOD for 5-HBC varied between 0.27 and 0.36 μg/L (ppb) and the LOQ was either 0.66 or 0.86 μg/L (ppb). Procedures for storing plasma before analysis were not described.

No carbendazim, STB or 5-HBC was detected in any of the plasma samples assayed for these metabolites of benomyl. The study authors therefore re-analysed the plasma using a more sensitive tandem LC-MS/MS method, described in a separate report (Meuling, 2001). The LOD for carbendazim in plasma using this method was 0.1 μg/L (0.1 ppb). Some 93 plasma samples were available for re-analysis, having been stored at -20°C for approximately 1 yr between the first analysis and the second.

"Blank" urine samples were taken from each subject prior to their initial treatment. All urine voided over the 72 h post-treatment period was collected over 0-24, 24-48 and 48-72 h after the commencement of treatment. To control for the potential effects of storage and transport of collected urine samples, "field spikes" (blank urine samples fortified with 5,6-DHBC-G or STB) were dispensed to the subjects, who were instructed to store and transport the sample in the same way as urine samples collected at home. Collected urine was returned to the laboratory 2 or 3 days post-exposure, urinary volume was determined by weighing and an aliquot of each sample was stored at -20°C prior to analysis. Urinary 5-HBC (for all subjects) and STB (for 1 subject) concentrations were measured by HPLC. The LOD and LOQ for 5-HBC in urine were 0.30 and 0.73 μg/L, respectively and the corresponding values for STB were 1.6 and 3.7 μg/L.

The dosing suspensions, glass applicator and cotton swabs used to remove the test compound were analysed for carbendazim by HPLC. The potentially absorbed dose (PAD) of benomyl was estimated by subtracting the amount removed by washing, from the dose applied.

Results

All subjects completed the study and did not show any treatment-related signs or symptoms. The average benomyl dose applied was 29.39 mg (SD = 0.84, range 28.05 – 30.51 mg). The average amount of benomyl washed off the skin after 4 h was 23.2 mg (SD = 2.02, range 19.6 – 27.4 mg). The average potentially absorbed dose was therefore 6.21 mg (SD = 2.41, range 0.65 – 10.54 mg), amounting to 21.0% of the applied dose (SD = 8.0, range 2.3 – 35.0). When estimated by this method, the dose potentially absorbed from the two larger exposure areas was approximately 25% higher than from the 300 cm^2 area (22.4 and 23.5% vs. 15.0%).

Analysis of urine

The assayed concentration of 5-HBC and STB in "field spike" urine samples lay between 99 and 110 μg/L. Assayed metabolite levels were not corrected for losses associated with transport and storage.

No STB was detected in the urine samples analysed for this metabolite. However, 5-HBC was detected in the post-treatment urine of all subjects, the "blank" (pre-study) urine samples from Subjects 5 and 8, and in urine samples voided by some other immediately prior to their repeat exposure. Table 14 shows the concentrations of 5-HBC detected in pre-exposure urine samples.

Table 14: The concentration of 5-HBC in urine of human subjects immediately prior to dermal application of benomyl

Subject	**3**	**3**	**4**	**5**	**6**	**7**	**8**	**8**
Date	13/12/99	22/12/99	20/12/99	7/12/99	14/12/99	14/12/99	7/12/99	14/12/99
5,6-DHBC-G (μg/L)	0.42	3.01	0.49	2.18	0.52	0.78	0.45	1.64

Note: 5-HBC was not detected in any other pre-exposure urine samples (LOD = 0.30 μg/L). The mean pre-exposure concentration of 5,6-DHBC-G in urine was 0.67 μg/L (N = 16; calculation treats samples in which 5-HBC was not detected as containing 0.5 x LOD = 0.15 μg/L).

Post-exposure, the highest urinary levels of 5-HBC were present during the first 24 h after the test material was applied, with the exception of two subjects, who excreted 5-HBC mainly during the 24 – 48 h period (Table 14). Compared with the pre-exposure levels, the concentration of 5-HBC over 0 – 24 h post-exposure was one or two orders of magnitude higher, averaging 16.17 μg/L and ranging between 4.33 and 43.24 μg/L. Mean excretion of 5-HBC decreased by approximately 50% on each successive day, but excretion was not complete in most subjects when urine sampling ceased at 72 h post-application. The results are summarised in Table 15.

Table 15: Urinary excretion of 5-HBC (μg, mean ± SD and range) by human subjects following dermal application of 30 mg benomyl (N = 16 exposures involving 8 subjects)

Time interval after commencement of exposure (h)		
0 –24	**24 – 48**	**48 – 72**
30.7 ± 27.6 7.3 - 95.4	17.0 ± 12.3 3.3 – 41.3	6.1 ± 3.6 0 – 11.5

The subjects excreted an average total of 53.8 μg of 5-HBC (SD = 39.9, range 12.4 – 146.7 μg) or 0.26 μmol (SD = 0.19, range 0.06 – 0.71 μmol) in their urine during the 72 h after treatment. When benomyl dose and 5-HBC excretion were expressed on a molar basis (correcting for differences between the molecular weights of benomyl and its metabolite), urinary excretion amounted to 0.26% (SD = 0.19, range 0.06 – 0.68%) of the applied dose, or 1.57% (SD = 1.50, range 0.22 – 5.97%) of the PAD. When the benomyl dose was applied over a 900 cm^2 area, approximately twice as much 5-HBC was excreted in the urine compared with the results obtained with the two smaller treatment areas. The correlation between application area and urinary 5-HBC excretion lay at the threshold of statistical significance ($p = 0.050$, Cochran-Manten-Haenszel test). However, there was no statistically significant relationship between application area and PAD or 5-HBC excretion as a percentage of the applied dose. Data are summarised in Table 16.

Table 16: Potentially absorbed dose (PAD) of benomyl and urinary excretion of 5-HBC in human subjects

Exposure area (cm^2)	Mean PAD (± SD) expressed as % of the applied dose	5-HBC excreted in urine (µg ± SD)	5-HBC in urine (% of applied benomyl ± SD)
300 (N = 4)	15.0 ± 11.1	36.3 ± 26.1	0.175 ± 0.118
600 (N = 4)	22.4 ± 8.52	30.6 ± 17.9	0.148 ± 0.084
900 (N = 8)	23.5 ± 4.56	74.1 ± 45.0	0.345 ± 0.210

Analysis of plasma

The second, more sensitive LC-MS/MS method yielded a deviation of 0 - 4.2% from QC standards, a repeatability of 3.7 – 6.5%, reproducibility of 4.2 – 12.4% and a recovery of 80 – 90%. QC samples of carbendazim in plasma were stored frozen (< -18°C) and thawed and assayed with each batch of experimental samples. The assay yielded a linear response over a concentration range of 0.1 – 50 µg carbendazim/L plasma.

Carbendazim was detected in 23 of the 93 plasma samples available for re-analysis. Results are summarised in Table 17. The highest plasma concentration of carbendazim was 1.037 µg/L. Most notable is the presence of carbendazim in the pre-dose samples from Subjects 1, 2, 3 and 6. In some cases, the pre-dose carbendazim level exceeded the concentrations detected following application of benomyl. In others, carbendazim was not detected in plasma until 2 – 4 h post-application, implying that there can be a significant delay between when benomyl makes contact with the skin and subsequent penetration. However, in Subject 3, a different pattern was observed, in which carbendazim was detected before and then intermittently after commencement of exposure. There is no biologically plausible mechanism to account for this finding. In the majority of replicates, carbendazim was present in final plasma samples taken 8 h after application and 4 h after removal of benomyl. The study authors acknowledged that the sampling period may have been too short to demonstrate the maximum concentration of carbendazim in plasma attainable following dermal exposure to benomyl. For these reasons, the OCSEH considers that the data on carbendazim levels in plasma are unsuitable for pharmacokinetic analysis.

Table 17: Carbendazim concentration (µg/L) in plasma of human subjects exposed dermally to benomyl

Sampling time (min)	Subject No., date and area of application site (cm^2)*						
	1 20/12/99 (300)	**2 13/12/99 (300)**	**2 20/12/99 (600)**	**3 13/12/99 (300)**	**3 22/12/99 (900)**	**6 14/12/99 (900)**	**8 7/12/99 (900)**
Pre-dose	0.116	1.037	ND	0.157	0.269	0.361	ND
30	0.106	ND**	ND	ND	0.227	ND	ND
45	ND	ND	ND	0.346	0.610	ND	ND
60	ND	ND	ND	ND	0.106	ND	ND
75	ND	ND	ND	ND	NA^	ND	ND
90	ND	ND	ND	ND	ND	ND	NA
120	ND	ND	ND	0.322	ND	ND	0.127
150	ND	ND	ND	ND	ND	ND	0.214
180	ND	ND	ND	ND	0.115	ND	NA
240	ND	ND	0.116	ND	0.248	0.127	0.109
480	ND	ND	0.161	0.399	0.123	0.203	0.111

*Plasma from Subjects 6 (600 cm^2 area) and 7 (900 cm^2 area) was also analysed, but carbendazim was not detected in any samples from these exposures.

**Not detected (LOD = 0.101 µg/L)

^Not available for re-analysis

Study authors' conclusions

The study authors concluded that dermal application of Benlate WP50 in humans leads to exposure [to benomyl] through dermal absorption [as evidenced by the detection of 5-HBC in urine and carbendazim in plasma], and that elimination of benomyl from the body is slower than expected [based on a previous study with carbendazim (Meuling *et al*, 1993)]. The study authors did not attempt to estimate the threshold dermal dose that would lead to detectable plasma levels of benomyl. They considered that testing of higher doses or larger surface areas in humans would not be possible due to ethical constraints.

Comment:

The above data should be interpreted with caution because of methodological failings. First, urine sampling ceased before most subjects had excreted their entire body burden of the metabolite 5-HBC. Second, benomyl metabolites were present in the urine and/or plasma from *all* subjects immediately prior to at least one of their dermal exposures. The insufficient urine sampling period would tend to cause under-estimation of the dose of benomyl that was absorbed, and is not amenable to correction. By contrast, "background" levels of carbendazim and/or 5-HBC would tend to cause over-estimation of the extent to which benomyl was absorbed dermally. However, comparison between pre- and post-exposure levels of 5,6-DHBC-G in urine suggests that the post-exposure excretion would have been over-estimated by less than 10%.

The presence of metabolites in pre-exposure blood and urine suggests that some subjects had been exposed to benomyl, carbendazim or thiophanate-methyl via the diet or from another source prior to the study. Meuling *et al* acknowledged this possibility, but considered that only "a few blanks and first blood (plasma) samples" would have been affected. Furthermore, it is also possible that the washout period between exposures was too short. The study authors did not correct their estimates of absorbed dose for the background excretion of 5-HBC in urine. The subjects' baseline daily excretion rate of 5-HBC could not be calculated, because only "spot" urine samples of unknown volume were obtained immediately prior to exposure. The OCSEH believes that any attempt to correct the results by subtracting the background concentration of 5-HBC in pre-exposure urine from the post-exposure data would merely introduce further errors of unknown magnitude and direction. Overall, the errors from background metabolite excretion and foreshortened urine sampling would tend to cancel each other out, and are likely to be significantly smaller than errors arising from inter-species extrapolation. Therefore, the data on urinary 5-HBC excretion may be used for estimating a dermal absorption factor for benomyl across human skin.

Estimation of the dermal absorption factor

The extent of benomyl absorption may be estimated from the relative proportion of the dose that would have been excreted in the urine. Meuling *et al* (1993) have reported that humans excrete an average of 15% of an iv dose of carbendazim via the urine over the first 30 h post-administration, in the form of 5-HBC. In the same study, human subjects also excreted an average of 15% of an oral dose of carbendazim via the urine as 5-HBC during the 48 h following treatment (graphical data from one volunteer suggested that almost all of the excreted 5-HBC appeared within the first 12 h). These findings suggest that the disposition of

benomyl in humans resembles the pattern observed in dogs, in which excretion occurs mainly via the faeces, rather than disposition in rats, in which urinary excretion predominates.

The mean dermal absorption in the group treated over the largest area (900 cm^2) is therefore estimated to be 0.345 x (100 ÷ 15) = 2.3% over 4 h at a loading of 0.033 mg benomyl/cm^2. However, the true value may have exceeded 2.3% because of unmeasured residence of benomyl/metabolites in the skin, extracellular fluid or body tissues. Hence, the estimated mean value of 2.3% over 4 h is probably conservative.

Readers will also note that there was a wide (11-fold) range between the lowest and highest proportions of the dose that appeared in the subjects' urine, implying significant inter-individual variation in absorption, metabolism or excretion of benomyl. The highest proportion of the applied dose excreted by any individual subject was 0.68% (Subject 8, first treatment on a 900 cm^2 area). The highest individual dermal absorption was therefore 0.68 x (100 ÷ 15) = 4.53% over 4 h. To account for heterogeneity within the human population, this value should be used for Occupational Health risk assessment. Assuming an 8 hour working day, the dermal absorption factor would be 9%.

Gooch JJ (1978) Fertility of workers potentially exposed to benomyl. DuPont de Nemours & Co. Wilmington, Delaware, USA. Report No. B/Tox 7 RE Dated October, 1978

and

Gooch JJ (1979) Fertility of workers potentially exposed to benomyl: II. Correlation with levels of exposure. DuPont de Nemours & Co. Wilmington, Delaware, USA. Report No. B/Tox 7 IS2 Dated April, 1979

This is a retrospective case-control study to determine the effect of benomyl on the fertility of 298 male manufacturing workers potentially exposed to benomyl between 1970 and 1977. 79% of the workers were aged between 19-64 years, while 78% of their spouses were aged 20-39. The duration of potential exposure ranged from less than one month to 95 months, but more than 51% of the workers had potentially been exposed for one to five months. The birth rates of the spouses of the exposed workers were compared with those of four control populations from the same state, region, and country (United States) (similarities between ethnical background and age were not specified). Results from this retrospective study found no reduction in fertility or birth rates of the study population who had been exposed to benomyl, even after stratifying the study population into groups with "average", "below average" and "variable" exposure to benomyl.

Unfortunately, there are many confounders associated with this case-control study. In particular the toxicological endpoint, namely fertility could not be clearly defined. The indirect method of determining adverse effects on fertility in this study does not take into account the differences in ethnical background or the willingness to procreate. Indeed, approximately 16% of spouses in 1973 (year of first recorded birth) were over 40 years, and less likely to conceive children after their peak reproduction years. The fact that the case-control birth rates were routinely less (at times significantly) than the birth rates of the workers potentially exposed to different concentrations of benomyl raises doubts about the ethnic and age comparability of the control groups. Even comparisons between the birth rates of potentially exposed workers with subsets of the control groups failed to remove the apparent increased fertility of the workers exposed to benomyl. Additionally, in the absence of a clear and tangible endpoint (such as reduced sperm count or incidence of birth defects), it is

difficult to assess the effect of benomyl on fertility or on birth rates. Thus no definite conclusion can be drawn from this study.

Dolk H et al (1998) Geographical variation in anophthalmia and microphthalmia in England, 1988-94. BMJ 317; 905 - 910

The study was initiated in response to public concerns arising from media reports of clusters of anophthalmia and microphthalmia in England, which postulated an association with exposure to benomyl. The authors intended to establish the presence or absence of any geographical variation in anophthalmia or microphthalmia, including large scale regional variation, excess prevalence in rural areas, and localised clustering. The analysis was based on a register of all such cases born in England in 1988 – 1994, which was established for the purposes of the study. The time period was chosen to overlap the period of concern and to collect enough cases for geographical analysis without risking under-ascertainment of cases in earlier years.

Some 444 cases were registered, excluding cases of trisomy of chromosome 13 and holoprosencephaly. Severity of microphthalmia was based on information supplied by clinicians in specially designed questionnaires. A subgroup of 237 severe cases was defined by excluding mild cases and 94 cases where the severity was unknown. A further subgroup of 189 severe cases of unknown aetiology was defined by excluding cases attributed to genetic origin and maternal infection. Postcodes of cases and all births were used for geographical localisation, and cases and births were grouped by regional health authority and district health authority; overall, there were 14 regions. In one case the region was unknown and in 14 cases the district was unknown. The urban or rural nature of the area of residence was determined by population density. Nine percent of births occurred in a rural setting. The state of socioeconomic deprivation of the district of residence was also assessed. The estimated prevalence of severe cases was calculated by assuming that the proportion of such cases among cases of unknown severity was the same as the proportion of severe cases among those of known severity.

The prevalence of anophthalmia and microphthalmia were compared by region, population density and socioeconomic deprivation by the Pearson and Armitage X^2 tests, adjusted where necessary for confounding using Poisson regression. Variation in underlying prevalence across regions and districts was estimated by the Martuzzi-Hills method, which removes the random sampling variation expected in small numbers. Expected numbers were calculated in each district of residence, stratified for region and population density quintile. Cuzick-Edwards and Diggle-Chetwynd tests were used to detect localised clustering by frequency among neighbours and by distance. The data included two pairs of siblings who strongly influenced the results at short distances. One of each pair was therefore removed from the clustering analysis on the basis that two members of a pair were probably not independent events.

The overall prevalence of anophthalmia and microphthalmia in England was 1.0 per 10000 births. Prevalence ranged from 0.6 per 10000 in the Mersey region to 1.6 per 10000 in the Oxford region, but regional variation in prevalence did not reach statistical significance ($p = 0.07$ for all cases and $p = 0.76$ for severe cases). There was no statistically significant heterogeneity in prevalence of all cases across districts ($p > 0.20$). The prevalence of anophthalmia and microphthalmia (all cases) increased with decreasing population density, ranging between 0.81 per 10000 (highest density quintile) to 1.45 per 10000 (lowest quintile).

The corresponding ranges for severe cases and severe cases of unknown aetiology were 0.54 – 1.29 and 0.40 – 0.97, respectively. The relative risk for all cases was 1.79 (95% CI = 1.15 – 2.81) in the most rural group compared with the most urban group, and for severe cases was 2.37 (95% CI = 1.38 – 4.08).

Socioeconomic deprivation had no effect on the prevalence of anophthalmia and microphthalmia ($p > 0.20$). There was no evidence of localised clustering, with respect to all cases or severe cases. However, within the two most rural quintiles, the observed number of severe cases of unknown aetiology within the three nearest neighbours (34) exceeded the number expected (26.4) by an amount approaching statistical significance ($p = 0.08$, Cuzick-Edwards test). The Diggle-Chetwynd test showed significant clustering (by distance) of severe cases of unknown aetiology in the Trent and Oxford regions and also in the most rural population density quintile, although the latter result was based on only one case-case pair in the Yorkshire region (no statistical values were presented). However, there was no significant clustering by this test when the two most rural quintiles were considered. Overall, the study authors considered that there was very little evidence for localised clustering.

The study authors considered that it was not possible to dismiss the gradient in prevalence from urban to rural areas, but cautioned that the evidence for a causal link between exposure to pesticides and anophthalmia and microphthalmia was weak, suggesting that other risk factors including maternal viral infection and hyperthermia were potentially more significant.

Spagnolo A et al (1994) Anophthalmia and benomyl in Italy: a multicenter study based on 940,615 newborns. Reprod Toxicol 8 (5); 397 – 403

and

Bianchi F et al (1994) Clusters of Anophthalmia: No link with benomyl in Italy. BMJ 308 (6922); 205 - 206

The study's objective was to evaluate the possible relationship between benomyl use and prevalence of anophthalmia and microphthalmia in newborns. Data from consecutive live births and stillbirths between January 1986 and December 1990 were obtained from the National Archive of Congenital Malformations (NACM), which covers 18 regions and approximately 1/3 of all Italian newborns. The analysis was performed after exclusion of malformations associated with chromosomal anomalies. Anophthalmia and microphthalmia cases were evaluated as a whole.

Data on the use of benomyl and thiophanate-methyl based pesticides for agricultural purposes between 1986 and 1990 were obtained from the Italian Annals of Agricultural Statistics. For each of the 18 regions covered by the NACM, a use rate was calculated by dividing the total kg benomyl used by the total number of hectares of ploughed land. Correlation between regional benomyl use rate and prevalence of anophthalmia and microphthalmia was performed using the Spearman non-parametric test. The 18 regions were divided into four groups based on increasing benomyl use, and analysis of data on anophthalmia and microphthalmia prevalence and benomyl use rate was performed by the chi-square test for trend. Confidence limits of 95% were derived from Poisson distributions for observed vs. expected ratios, and exact confidence limits were calculated using the Epi-Info software package.

A further analysis was carried out on the association between anophthalmia and microphthalmia prevalence and parental occupation, using a subset of 768005 newborns. Newborns with preauricular tags were used as controls, since the presence of this malformation has never been associated with pesticides in either humans or animals, according to the study authors. The prevalence of preauricular tags was constant among the different regions and over time. Association between pesticide use and parental agricultural occupation was also evaluated for children with isolated preauricular tags and for those with other isolated malformations. This analysis was based on the assumption that agricultural workers have a higher chance of being exposed to benomyl than the general population.

Out of 940615 births there were 33 and 78 cases of anophthalmia and microphthalmia, respectively, with an overall combined prevalence rate of 1.18 per 10000 births. Among the anophthalmia cases, 15 were isolated, 10 were associated with other malformations and a chromosomal anomaly was present in eight. Some 23 of the microphthalmia cases were isolated, 33 were associated with other malformations, and a chromosomal anomaly was present in 22. The prevalence of anophthalmia in 1986 was more than double that during the succeeding four years (0.74 per 10000 vs. 0.22 – 0.31, respectively), but there was little time-related variation in the prevalence of microphthalmia.

When cases associated with chromosomal anomalies were excluded, the overall prevalence rate of anophthalmia and microphthalmia was 0.86 per 10000. No cases occurred in Abruzzi, Basilicata and Sicilia, while the highest prevalence rate was 3.83 per 10000 in Marche (odds ratio of 4.45). However, interregional distribution was not statistically significantly heterogeneous ($X^2 = 18.61$, df = 17, p = 0.3).

Data were presented on the combined use of benomyl and thiophanate-methyl products, expressed in kg/ha plough land in each region. There was marked interregional variation in the mean annual use rate of benomyl and thiophanate-methyl over the 5 years of the study. The lowest mean value of 0.01 kg/ha was recorded in Val d'Aosta, while the highest (0.39 kg/ha) occurred in Trentino. When the data were averaged across all regions, the lowest and highest annual application rates were 0.09 and 0.21 kg/ha, recorded in 1990 and 1988, respectively. The overall five-year mean for all districts was 0.15 kg/ha/yr.

The regional prevalence of anophthalmia and microphthalmia was not significantly correlated with the use rate of benomyl/thiophanate-methyl (Spearman rho = -0.27, p = 0.26). There was no relationship between the annual prevalence and the use rate of benomyl / thiophanate-methyl in that year. Furthermore, when districts were compared, there was a significant trend (X^2 for trend of 4.17, p = 0.04) towards decreasing anophthalmia and microphthalmia prevalence with increasing benomyl/thiophanate-methyl use rate. Mean prevalence rate was highest (1.17 per 10000) in districts where annual use rate was < 0.1 kg/ha. Conversely, prevalence rate was lowest (0.63 per 10000) in districts where annual use rate was $\geq$ 0.3 kg/ha.

Information on parental occupation was available for 90 of the 95 anophthalmia and microphthalmia cases (including those associated with chromosomal abnormalities) occurring in the subset of 768005 newborns. Four of these 90 affected babies had one parent with an agricultural occupation; in two cases it was the father, and in the other two cases it was the mother. By comparison, 42/912 of the babies born with preauricular tags had at least one parent who worked in an agricultural setting. The odds ratio was 0.96 (95% CL = 0.25 – 2.75). When deformities associated with chromosomal abnormalities were excluded, the odds

ratio remained non-significant at 0.68 (95% CL = 0.08 – 2.72). Thus, the study did not demonstrate any association between anophthalmia and microphthalmia and parental occupation.

The study authors concluded that there was no positive association between benomyl use and the prevalence of anophthalmia and microphthalmia, and that agricultural workers do not appear to have a greater proportion of children with eye deformities.

Kristensen P et al (1997) Birth defects among offspring of Norwegian farmers, 1967-1991. Epidemiology 8 (5); 537 – 544

and

Kristensen P & Mirgens L (1994) …or in Norway BMJ 308 (6922); 205 – 206

In the main study, the authors examined the hypothesis that there was an association between parental pesticide exposure and birth defects of the CNS (in particular the neural tube), orofacial clefts, limb reduction, cryptorchidism and hypospadias. The second citation considered here is a letter from the same research team to the editor of the BMJ, which specifically addresses the possibility of an association between eye defects and benomyl use within the main study cohort.

The study population consisted of 149,254 farm holders and 104,370 spouses who were linked to the Medical Birth Registry of Norway to identify a total of 192417 "farmers' births" between 1967 and 1991. An external reference population was established, consisting of all births (totalling 61351) to non-farmers in agricultural municipalities. Pesticide exposure indicators were derived from information obtained in national agricultural and horticultural censuses. The indicators chosen were the amount of money spent on pesticides in 1968 and tractor pesticide spraying equipment on the farm in 1979. The use of phosphorus and nitrogen fertiliser per ha was quantified. Additional information included the commodities produced by the farm (i.e. animals, grain, field vegetables or orchards / greenhouses), water source, and annual hours of parental work input on the farm.

Prevalence at birth was calculated for total and specific birth defects and relative prevalence was approximated using odds ratio estimates from contingency tables. Exposed groups were compared with unexposed groups; for combinations of exposures, the group with neither of the exposure characteristics served as referent. Contingency tables were stratified according to potential confounding variables (including year of birth, geographical distribution, maternal age, parental consanguinity and birth order), which were controlled for by fitting logistic regression models.

To allow for seasonal fluctuation in pesticide use and exposure, data were examined by calendar quarter of conception, on the assumption that birth defects were caused by parental exposure during the three months prior to conception or during pregnancy. Secular trends in the associations between pesticide indicators and specific defects were investigated in separate analyses in 5-yr strata of births. This allowed for temporal changes in the use of different pesticides (in particular the withdrawal of organochlorines in Norway from about 1970).

Benomyl was introduced in Norway in 1971, where it had uses in greenhouses, orchards, field vegetables (during spring) and grain (during late autumn). The study authors commented that benomyl was a minor pesticide, the maximum annual sale [to 1994] being 1682 kg of active constituent in 1980. Special examinations were performed on data from children conceived throughout the year (farms with greenhouses), children conceived in April – June (farms with orchards), children conceived in January-April (farms with field vegetables) and children conceived in August – November (grain farms).

Some 4565 birth defects were observed in 4189 offspring among the 192417 farmers' births. Total prevalence at birth in the study population was similar to prevalence in the non-farming population. Similarly, prevalence of CNS defects and defects of the eye and most other organ systems were not greater in the farming population (Table 18). However, the study authors considered that the OR estimates for respiratory system defects, oesophageal atresia and pigmented nevi were elevated in the study population (ORs for these conditions were 1.85, 1.87 and 1.55, respectively).

Table 18: Prevalence of selected birth defects in offspring of farmers and non-farmers

Birth defect	Prevalence/10000 (farmers)	Prevalence/10000 (non-farmers)	Adjusted* OR and 95% CL
Total	218	231	1.02; 0.96 – 1.09
CNS	14.9	14.7	1.10; 0.73 – 1.20
Anencephaly	4.4	3.6	1.10; 0.68 – 1.79
Spina bifida	5.0	6.2	0.76; 0.51 – 1.13
Hydrocephaly	4.2	4.4	0.94; 0.60 – 1.50
Eye (all defects)	1.4	2.6	0.49; 0.25 – 0.98
Face	5.6	4.6	1.22; 0.78 – 1.90
Isolated cleft palate	4.8	5.2	0.93; 0.60 – 1.43
Total cleft lip	14.8	15.6	0.94; 0.74 – 1.20

*Adjusted for confounding variables comprising year of birth, maternal age, geographical region and parental consanguinity.

Moderate increases in risk for spina bifida and hydrocephaly were found in the subgroups having possible exposure to pesticides in orchards or greenhouses [spina bifida: 5 exposed cases, OR = 2.76; 95% CL = 1.07 – 7.13] [hydrocephaly: 5 exposed cases, OR = 3.49; 95% CI = 1.34 – 9.09]. Exposure to pesticides, in particular in grain farming, was also associated with limb reduction defects [OR = 2.50; 95% CI = 1.06 – 5.90]. However, these and other findings are not directly relevant to this review and will not be considered further here.

Four of the 192416 farmers' children had been diagnosed with anophthalmia or microphthalmia, yielding a crude prevalence of 0.21 per 10000 newborns. By comparison, this was half the crude prevalence (0.43 per 10000) in the total Norwegian population born in 1981 – 1991. In all four cases the infants were singletons born alive at full term. One had additional [unidentified] birth defects. The four families lived in different parts of Norway, with no temporal clustering of the pregnancies (conceptions between 1971 and 1988). None of the families had a greenhouse or orchard, but one child with microphthalmia came from a farm that grew field vegetables, and therefore had parents who were considered as being potentially exposed to benomyl. Given that 11% (21843) of the entire cohort of farmer's children were considered as having been potentially exposed to benomyl, the crude prevalence rate of microphthalmia / anophthalmia among children from this group was 1 per 21843 (=0.46 per 10000), which is similar to the prevalence for the entire population.

Overall, there was no evidence that parental exposure to pesticides was associated with eye defects including anophthalmia and microphthalmia in their offspring, or that anophthalmia and microphthalmia were more prevalent among children whose parents lived or worked on farms where benomyl may have been applied.

PART II: OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

1. PRODUCTS AND THEIR USE PATTERNS

At the commencement of this review, there were 21 carbendazim products registered in Australia (Table 47) The labels of all carbendazim products were suspended from 4 May 2007 until 4 May 2009, with product supplied after 4 May 2007 required to have additional instructions securely affixed to the container, advising the risk of potential birth defects to women of childbearing age who may come in contact with carbendazim products. Fourteen products had an extension of the suspension from 1 June 2009 until 4 May 2010, and an additional eight were no longer suspended as the labels were approved after May 2007 and contained the necessary instructions (Commonwealth of Australia 2009[14]).

Carbendazim products

At the commencement of this review there were 21 registered products containing carbendazim, 19 were SC formulations (17 containing 500 g/L carbendazim and two containing 80-100 g/L), one was an EC (75 mg/L carbendazim) and one was a wettable powder (WP 500 g/kg carbendazim). As of December 2009, there are 18 registered products, including the addition of one 500g/L SC formulation product and the loss of five SC 500 g/L products, however only the original 21 registered products are included in this review. Of those products, the 17 SC 500 g/L and the WP 500 g/kg carbendazim products are intended for use in agricultural situations in spray and dip applications for the control of fungal diseases on food plants, fruits, ornamentals, pasture and turf. The application rates depend on the crop and the disease being treated, with typical application rates being 40-50 mL/100L water for post-harvest treatment of fruit, 25-50 mL/100L water for spraying until petal fall, 40-100 mL/100L water for high volume spraying, 300-550 mL/ha for low volume spraying (including pasture), 65 mL/100L water for treatment of sugar cane seed pieces, 200 mL/100 L water for treatment of ginger seed pieces and 60 mL/100 m^2 for turf (Table 47).

The two SC products containing 80-100 g/L and the EC 75 mg/L carbendazim products are intended for use by timber producers as timber preservatives. They can be applied as a dip or as a spray treatment. Recommended dilution rates are 200-800 mL/100 L water, depending on location and conditions and on the length of storage of the treated timber. Public and occupational health and safety assessments for the two SC products (Hylite 80 Anti-Sapstain containing 80 g/L oxine-copper plus 80 g/L carbendazim and Antiblu CC Concentrate Timber Fungicide containing 450 g/L chlorothalonil plus 100 g/L carbendazim) as well as the EC product (Hylite Timber Preservative containing 90 g/L zinc naphthenate plus 75 g/L carbendazim) have previously been conducted by OCSEH. The existing PPEs recommended for these three products are reconsidered in this assessment under the Safety Directions section, but otherwise an OHS assessment is not considered necessary. The OHS risk assessment will, therefore, only be conducted only for the other 18 products which includes 17 SC 500 g/L carbendazim products and one WP 500 g/kg carbendazim product. None of these products are marketed for home and/or garden use.

[14] Commonwealth of Australia Gazette, No. S 97, 3 June 2009: Special Gazette.
This label suspension was replaced with a further suspension of products in January 2010 that required labels to have additional instructions applied that provided the signal heading appropriate for S7, extended the birth defect warning to include a warning regarding irreversible infertility in male laboratory animals and, for the horticultural products, included the restraint statement "DO NOT apply to turf, grapes, cucurbits (including melons), citrus fruit, custard apple, mango, pome fruit (apples or pears) or stone fruit (including cherries).

Table 47: Carbendazim products registered in Australia

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APVMA Product Code	Product Name	Registrant	Carbendazim concentration
Carbendazim products			
30399*	BASF Bavistin FL Systemic Fungicide	BASF Australia Ltd	500 g/L
30740#	Hylite Timber Preservative	Osmose Australia Pty Ltd	75 g/L
47708#	Hylite 80 Anti-Sapstain	Osmose Australia Pty Ltd	80 g/L
50528	4Farmers Carbendazim 500 Fungicide WP	4 Farmers Pty Ltd	500 g/kg
51514	Antiblu CC Concentrate Timber Fungicide	Kopers Arch Wood Protection (Aust) Pty Ltd	100 g/L
52878#	Farmoz Howzat SC Systemic Fungicide	Farmoz Pty Ltd	500 g/L
53061	Boomer Systemic Fungicide	Sipcam Pacific Australia Pty Ltd	500 g/L
53390	Chemag Carbendazim 500 SC Fungicide	Imtrade Australia Pty Ltd	500 g/L
53587#	Campbell Goldazim 500 SC Systemic Fungicide	Colin Campbell (Chemicals) Pty Ltd	500 g/L
54167	Kendon Carbendon SC Systemic Fungicide	Kendon Plant Care Pty Ltd	500 g/L
54269*	Nufarm Carbend Fungicide	Nufarm Australia Ltd	500 g/L
55949*	Rotate SC Systemic Fungicide	Kendon Chemicals & MNFG Co Pty Ltd	500 g/L
56497*	Sava 500 Fungicide	Allfire Enterprises Pty Ltd (now Agvantage)	500 g/L
56692	Superway Carbendazim 500 Systemic Fungicide	Superway Garden Ag & Pest Products Pty Ltd	500 g/L
56783	Halley Carbendazim 500 Systemic Fungicide	Halley International Enterprise (Australia) Pty Ltd	500 g/L
58452	Kenso Agcare Carbendazim 500 SC Systemic Fungicide	Kenso Corporation (M) SDN BHD	500 g/L
58832*	Conquest Commodore 500 Fungicide	Conquest Agrochemicals Pty Ltd	500 g/L
58886#	Crop Care Bavistin FL Systemic Fungicide	Crop Care Australasia Pty Ltd	500 g/L
59434	Shincar 500 SC Fungicide	Sinon Australia Pty Ltd	500 g/L
59815#	Nufarm Spin Flo Systemic Fungicide	Nufarm Australia Ltd	500 g/L
60942	Ospray Carbendazim 500 Fungicide	Ospray Pty Ltd	500 g/L

*Not registered on PUBCRIS December 2009

Product not included in the extended suspension 1 June 09-4 May 10 as their labels were compliant with the suspension instructions. Also excluded from the extended suspension were Product No. 61334 '4Farmers Carbendazim 500 SC Fungicide' and Product No. 63167 'Country Carbendazim 500 Fungicide', which were not included in this review and had labels that complied with the requirements of the May 2007 suspension..

Table 48: Use patterns for carbendazim products

Fruits, vegetables, pasture and roses

Crop	Pest	Product description	Maximum rate	Critical comments
Chickpeas, faba beans, lentils and vetch	Chocolate spot (*Botrytis fabae*), grey mould (*Botrytis cinerea*)	500g/L SC	500mL/ha	Apply a maximum of two consecutive applications at 14 day intervals. Apply in a minimum of 100L water per hectare.
Cucurbits	Powdery Mildew (*Sphaerotheca fuliginea*)	500g/L SC 500g/kg WP	50mL/100L or 550mL/ha 50g/100L or 500g/ha	Begin application when disease first appears, repeat at 7 to 14 day intervals. Use the higher rate and shorter intervals when disease pressure is high and plants are growing rapidly.
Pasture	Clover scorch (*Kabatiella caulivora*) Cercospora *(Cercospora zebrina)*	500g/L SC	550mL/100L plus 1L/100L summer spray oil	Apply at 'closing up' of pasture in a minimum spray volume of 150 L/ha. Repeat 30 days later if there is a build up of disease. Use the higher rate if disease is well established at 'closing up', repeat at this rate 30 days later if disease continues to develop.
Red clover, Subterranean clover	Clover scorch (*Kabatiella caulivora*) Cercospora *(Cercospora zebrina)*	500g/L SC 500g/kg WP	550mL/100L plus 1.5L/150L summer spray oil 50g/100L plus 1.5L/150L petroleum oil	Apply at 'closing up' of pasture in a minimum spray volume of 150 L/ha. Repeat 30 days later if there is a build up of disease. Use the higher rate if disease is well established at "closing up", repeat at this rate 30 days later if disease continues to develop.
Roses	Powdery mildew (*Oidium* or *Sphaerotheca* spp.)	500g/L SC 500g/kg WP	25mL/100L plus 1L summer oil/100L 25g/100L plus 1L summer oil/100L	Begin application when disease first appears and repeat at 7 to 14 day intervals throughout the growing season. Shorten intervals during humid weather.
	Black spot *(Diplocarpon rosea)*	500g/L SC 500g/kg	50ml/100L 50g/100L	
Strawberries	Grey mould (*Botrytis cinerea*)	500g/L SC 500g/kg WP	50mL/100L 50g/100L	Begin application when disease first appears or at flowering and repeat at 7 to 14 day intervals. Use higher rate and shorter intervals when disease pressure is high.
Turf	Dollar spot (*Sclerotinia homoeocarpa*)	500g/L SC	60mL/100m2	Apply in sufficient water to give good coverage. Commence application at beginning of damp weather and repeat at intervals of 4 weeks.

Tree and vine crops

Crop	Pest	Product description	Maximum rate	Critical comments
Apples	Powdery Mildew (*Podosphaera leucotricha*)	500g/L SC 500g/kg WP	50mL/100L (1L/ha) 50g/100L	Apply at 7 to 10 day intervals until petal fall. Use higher rate when disease pressure is high.
	Black spot (Scab) (*Venturia inaequalis*)			
Custard apples	*Cylindrocladium* spp., *Pseudocercospora* spp.	500g/L SC	50mL/100L	DO not apply in tank mixes with products containing Copper oxychloride. Apply a maximum of 4 sprays. First spray to be applied at fruit set after flowering. Where disease has occurred previously apply a second spray 2 – 4 weeks later. If high disease pressure should occur, a further two sprays may be applied. All sprays must be applied at a minimum interval of 14 days.
Grapes	Grey mould (Bunch rot) (*Botrytis cinerea*)	500g/L SC 500g/kg WP	100mL/100L or 1.1L/ha 100g/100L or 1.1kg/ha	Apply at early flowering, 80 to 100% capfall and pre-bunch closure. Further applications may be necessary at veraison and pre-harvest, if wet weather favours infection. Application should be made in sufficient water to obtain thorough coverage of the crop. High volume application should be made in sufficient water to obtain thorough coverage of the crop. For applications close to harvest this would require a minimum of 1100 L/ha. For low volume application, the spray pressure should be high enough to ensure penetration of the leaf canopy and coverage of the bunches.
Macadamia nuts	Macadamia husk spot (*Pseudocercospora* spp.)	500g/L SC	50mL/100L (1L/ha) plus wetting agent at 100mL/100L	Apply at 5 and 8 weeks after main flowering – stage 2 anthesis (white flowering stage). Remove any fallen nuts from under trees prior to spraying. DO NOT apply more than 2 applications per season.
Pears	Black spot (Scab) (*Venturia pirina*)	500g/L SC 500g/kg WP	50mL/100L (1L/ha) 50g/100L	Apply at 7 to 10 day intervals until petal fall. Use higher rate when disease pressure is high.
Stone fruit	Blossom blight (*Monilinia fructicola*)	500g/L SC 500g/kg WP	50mL/100L (1L/ha) 50g/100L	Apply at pink or white bud stage, 10% blossom and petal fall. Apply the higher rate when disease pressure is high.
	Brown rot (*Monilinia fructicola*)	500g/L SC 500g/kg WP	40mL/100L 40g/100L	Apply 3 and 1 week prior to harvest following earlier application of blossom blight sprays.

Post-harvest uses

Crop	Pest	Product description	Maximum rate	Critical comments
Apples	Blue mould (*Penicillium expansum*)	500g/L SC 500g/kg WP	50mL/100L 50g/100L	Submerge fruit for approximately 30 seconds. Dipping should occur no later than 24 hours after harvest. Top up dip at the recommended rate of 50mL/100L. TAS only: Always apply the treatment whenever the apples are to be dipped in diphenylamine prior to storage.
Bananas	Crown rot (*Colletotrichum musae*)	500g/L SC 500g/kg WP	40mL/100L 40g/100L	Submerge fruit for approximately 30 seconds.
Citrus	Blue and green moulds (*Penicillium* spp.)	500g/L SC 500g/kg WP	100mL/100L 100g/100L	Submerge fruit for approximately 30 seconds.
Mangoes	Anthracnose (*Colletotrichum* spp.), Stem end rot (*Dothiorella* spp.)	500g/L SC	100mL/100L	Submerge for approximately 5 minutes at 52°C.
Pears	Blue mould (*Penicillium expansum*)	500g/L SC 500g/kg WP	50mL/100L 50g/100L	Submerge fruit for approximately 30 seconds. Dipping should occur no later than 24 hours after harvest. Top up dip at the recommended rate of 50mL/100L.
Rockmelons	Fusarium fruit rot (*Fusarium* spp.), Sour rot (*Geotrichum candidum*), Alternaria fruit rot (*Alternaria* spp.), Rhizopus soft rot (*Rhizopus stolonifer*), Pink mould rot (*Trichothecium roseum*)	500g/L SC	100mL/100L plus 130mL Panoctine plus 10mL Chemwet 1000/100L water	Dip fruit for 45 seconds within 24 hours of harvest.
Stone fruit	Brown rot (*Monilinia* spp., *Sclerotinia* spp.)	500g/L SC 500g/kg WP	100mL/100L 50g/100L	Submerge fruit for approximately 30 seconds. Use higher rate when disease pressure is severe or when longer term storage is required.

Pre-planting uses

Crop	Pest	Product description	Maximum rate	Critical comments
Ginger seed pieces	Rhizome / seed piece rot (*Fusarium* spp.)	500g/L SC 500g/kg SC	200mL/100L 200g/100L	Cut seed pieces to desired length from rhizomes free of rot. Submerge for 5 minutes and allow to dry before planting.
Sugar cane	Pineapple disease (*Ceratocystis paradoxa*)	500g/L SC 500g/kg	65mL/100L 125ml/200L 125g/200L	Apply to cut seed pieces as a dip or spray so as to obtain thorough wetting. After dipping allow to drain. When replenishing dip, top up with 65 mL (125mL or 125g) in 100L water.

Timber preservative

Timber	Pest	Product description	Maximum rate	Critical comments
Sawn lumber. Normal conditions. Winter or short stock piling	Sap stain and mould	75g/L EC	6L/1000L	Freshly sawn lumber should be treated as soon as possible after processing.
Sawn lumber Severe conditions. Summer or long stock piling			8L/1000L	Higher rates recommended for mould control.
Round wood / Poles			8L/1000L	Poles should be treated immediately after peeling or shaving.
Boron bath			6L/1000L	Ensure timber is completely wetted by treatment solution.
Sawn lumber. Normal conditions. (temperate conditions and low humidity).		80g/L SC	6L/1000L (dip) or 100L/1000L (spray)	Dip Applications: Completely disperse the concentrate in a small amount of water before addition to the final mix solution. Spray Applications: Spray application solution strengths are in the range of 60L – 100L per 1000L.
Sawn lumber. Severe conditions. (warm to hot temperature and high humidity)			8L/1000L (dip) or 100L/1000L (spray)	
Sawn lumber. export conditions. (hot temperatures in shipping and high humidity)			10L/1000L (dip) or 100L/1000L (spray)	
Poles and rounds			8L/1000L	
Timber – Pine (freshly sawn	Sap stain, mould and decay fungi	100g/L SC	800mL/100L	Product may be applied by dip or spray treatment. Ensure all surfaces are thoroughly covered with the solution. Use the lower rate for short term storage in cool or dry conditions or where timber is strip stacked. Use the higher rate for longer storage or where conditions are warm and humid and timber is block- stacked.

2. TOXICOLOGICAL HAZARDS OF CARBENDAZIM

The toxicological hazards of carbendazim have been addressed in the concurrent toxicological and public health assessment of carbendazim (see Part I).

Toxicological endpoints and NOELs for OHS risk assessment

Workers may be exposed to carbendazim through dermal contact with the undiluted product, the spray mixture or treated vegetation. In addition, exposure may occur via inhalation of spray mist. Therefore, a dermal NOEL and inhalation NOEL are the ideal basis for the worker risk assessment of carbendazim. Table 50 summarises the NOELs/LOELs from toxicity studies performed in laboratory animals deemed suitable for OHS risk assessment purposes (noting that there were no suitable human studies in the database).

The most sensitive toxicological endpoints for carbendazim following repeated dosing were foetal malformations and testicular toxicity observed in rats and these are therefore the most appropriate toxicological endpoint for OHS risk assessment purposes.

Dermal NOEL

Normally, dermal repeat-dose studies form the optimal basis for setting a dermal NOEL for risk assessment purposes. Table 50 contains a 21-day dermal repeat-dose toxicity study performed with carbendazim in rabbits, which has a NOEL of 10,000 mg/kg bw/d (Fave 1981). However, this study did not investigate the relevant toxicological endpoints and hence can not be used. In two oral developmental toxicity studies in rats (Hofmann & Peh 1987a; Alvarez 1987), there was an increased incidence of foetal malformations affecting the head, spine and ribs as well as reduced foetal weight at doses of 20-30 mg/kg bw/d in the absence of maternal toxicity. The NOEL for these effects was 10 mg/kg bw/d.

In a rat reproduction study (Gray *et al* 1990), effects on sperm production and morphology were observed at the lowest dose (50 mg/kg bw/d). This study is supported by effects noted in two separate single dose studies in rats where doses of 50 mg/kg bw/d resulted in effects on the testis, including premature release of immature germ cells, atrophy and decreased growth of the seminiferous tubules, and increased frequency of micronuclei in spermatids (Nakai *et al* 1992; Matsuo 1999). This LOEL is considered the most appropriate for OHS risk assessment purposes. The OHS NOEL is therefore 5 mg/kg bw/d, incorporating a safety factor of 10 to account for the use of a LOEL.

Route-to-route extrapolation usually involves a consideration on the internal dose. In the case of oral-to-dermal extrapolation, this consideration takes into account the absorption across the GI tract following oral administration. For carbendazim, the extent of absorption was estimated to be approximately 85% in rats (Meuling *et al* 1993). Therefore, an adjustment of the NOEL is not required. The acceptable margin of exposure (MOE) is ≥ 100, resulting from application of a 10-fold uncertainty factor for inter-species extrapolation and 10-fold factor for intra-species variability.

Table 50: Summary of NOELs relevant for OHS assessment for carbendazim products

Species (study type, route)	NOEL (mg/kg bw)	LOEL (mg/kg bw)	Toxicological Endpoint	Reference
Short-term studies				
Rabbits (21-day, dermal)	10000	-	No effects (testes not examined)	Fave (1981)
Developmental studies				
Rats (developmental, gavage)	30 (dams) 10 (foetuses)	60 (dams) 30 (foetuses)	Dams: Reduced maternal bodyweight gain. Foetuses: Reduced foetal weight and increased incidence of malformation.	Hofmann & Peh (1987a)*
Rats (developmental, gavage)	20 (dams) 10 (foetuses)	90 (dams) 20 (foetuses)	Dams: Reduced maternal bodyweight gain, increased liver weight. Foetuses: Reduced foetal weight.	Alvarez (1987)*
Rats (developmental, diet)	747 (dams & foetuses)	-	No effects at highest dose tested.	Culik *et al* (1970)*
Rabbits (developmental, gavage)	20 (dams) 10 (foetuses)	125 (dams) 20 (foetuses	Dams: Reduced maternal bodyweight gain and increased abortion. Foetuses: Reduced implantation and live litter size, increased resorption	Christian *et al* (1985)*
Effects on male reproductive tract				
Rat (single oral dose, study on effects on male reproductive tract, 70 day observation)	-	50	Premature release of immature germ cells, atrophy of seminiferous tubules, decreased seminiferous tubules, abnormal growth of efferent ductules. Effects persisting for at least 70 days.	Nakai *et al* (1992)
Rat (single oral dose, study on induction of micronucleus formation in spermatids)	-	50	Increased frequency of micronuclei in spermatids	Matsuo (1999)
Mice (study on effects on germ cells, 5 days, gavage)	500	1000	Altered ratio of testicular cell types present, abnormal sperm head morphology, and altered sperm chromatin structure	Evenson *et al* (1987)
Rat (reproduction, gavage)	-	50	Rats: Mild testicular atrophy, ↓ sperm, altered semen quality and sperm morphology.	Gray *et al* (1990)
Hamster (reproduction, gavage)	-	400 (only dose tested)	Hamsters: 16-17% lower sperm count	
Rat (30-day range-finding study, diet)	200	1000	Inhibition of spermatogenesis, reduced bodyweight	Scholz & Weigand (1972)*
Rat (93 days, diet)	163	780	Small testes and atrophy of seminiferous tubuli, reduced bodyweight	Scholz & Schultes (1973)*
Rat (2 years, diet)	125	250	Diffuse testicular atrophy, prostatitis	Sherman (1972)
Dog (2 years, diet)	7.5	125	Atrophic tubules of the testes, prostatitis	Reuzel *et al* (1976)

* Reproduced from JMPR (2005) report. Original data not independently assessed by OCSEH.

Inhalational NOEL

Since there are no available repeat-dose inhalational toxicity studies, the NOEL used for the dermal OHS risk assessment will also be used for the inhalational OHS risk assessment of carbendazim.

Dermal absorption factor

The rate of dermal absorption of carbendazim across human skin is unknown. However, dermal absorption factor across human skin for benomyl has been estimated from the relative proportion of the dose that had been excreted in the urine (Meuling 2000; 2001). These studies have reported that the highest proportion of the applied dose of benomyl excreted in the urine by any individual subject was 0.68% over the following 72 hours (on a 900 cm^2 treated area for 4 hours). The value of 0.68% certainly underestimates the proportion of benomyl absorbed, given that humans excrete only 15% of an intravenous or oral of dose of carbendazim (Meuling *et al* 1993). Therefore, a 6.66-fold factor (100 ÷ 15) should be applied to the excretion value of 0.68%, yielding a dermal absorption factor of 4.5% over 4 hours. Extrapolating this to the standard 8-hour work day equates to a dermal absorption factor of approximately 9%. Based on similarities in toxicological effects and chemical structure, this dermal absorption factor was considered appropriate and thus will be used in the OHS risk assessment for carbendazim.

Inhalation absorption factor

Since there is no data available, a default 100% inhalation absorption factor is assumed for carbendazim.

4. ASSESSMENT OF EXPOSURE AND RISK -OCCUPATIONAL

4.1 Estimation of occupational exposure and risk during mixing/loading and application

Since no exposure data are available, estimates of occupational exposure to and of risk from SC 500 g/L and WP 500 g/kg carbendazim products have been prepared utilising exposure modelling using the Pesticide Handlers Exposure Database (PHED) and the UK Predictive Operator Exposure Model (POEM). The MOE, calculated from PHED and POEM data, are presented in the Tables 50 and 51. In the absence of specific information for carbendazim products, the OCSEH used the following assumptions based on previously available information:

Assumptions

- 6 hour application period per day plus 2 hour mixing/loading
- Workers wear long pants and long-sleeved shirt
- Dermal absorption of carbendazim of 9%
- Inhalation absorption of carbendazim of 100%
- 70 kg bodyweight for an average worker
- OHS NOEL of 5 mg/kg bw/d from a rat reproduction study.
- Transmission across chemical-resistant clothing 5% (Thongsinthusak 1993)
- Transmission across overalls 10% (Thongsinthusak 1993)
- Protection afforded by a dust/mist respirator 80% (PHED Surrogate Exposure Guide 1998)

Estimates have been generated for the following situations:

Situation 1 – Open mixing and loading of liquids (PHED scenario 3) and wettable powders (PHED Scenario 4).

Situation 2 - Application by ground boom spray (pastures and field crops) (PHED scenario 13).

Situation 3 - Application by airblast (orchard crops) (PHED scenario 11).

Situation 4 - Application by hand-held equipment (ornamental crops, turf) (PHED scenario 20-knapsack, and POEM scenario 5-vehicle mounted spray tank).

Situation 5 - Preparation and use as a dip.

In the absence of information, the following assumptions have been applied for daily work rates for workers preparing and applying the SC 500 g/L and WP 500 g/kg carbendazim products.

Table 51: Work rates assumed for PHED/POEM calculations

Situation	Work rate (ha/day)	Maximal application rate (kg/ha)	Carbendazim applied (kg/day)
Boom spray	50	0.5	25
Airblast application	30	1.1	33
Manual application (knapsack)	0.2	3	0.6
Manual application (vehicle-mounted tank)	1	3	3

Table 52: Exposure during mixing/loading/application of SC 500 g/L and WP 500 g/kg carbendazim products

Estimate	*Gloves*	Systemic exposure to carbendazim (mg/kg bw/d)*				
		Mixer/Loader Dermal	*Applicator Dermal*	*Mixer/Loader Inhalation*	*Applicator Inhalation*	*Total exposure*
(PHED scenario 3): All liquids, open mixing/loading	N	0.26	NA	0.0012	NA	0.26
	Y	0.002	NA	0.0012	NA	0.003
(PHED scenario 4): Wettable powders, open mixing/loading	N	0.35[1]	NA	0.045	NA	0.40
	Y	0.016	NA	0.045	NA	0.061
(PHED scenario 3 and 13): All liquids, open mixing/loading, and groundboom application, open cab	N	0.2	0.001	0.0009	0.0006	0.2
	Y	0.0016	0.001	0.0009	0.0006	0.004
(PHED scenario 4 and 13): Wettable powders, open mixing/loading, and groundboom application, open cab	N	0.26[1]	0.001	0.034	0.0006	0.30
	Y	0.012	0.001	0.034	0.0006	0.047
(PHED scenario 3 and 11): All liquids, open mixing/loading, and airblast application, open cab	N	0.26	0.034	0.0009	0.0046	0.30
	Y	0.002	0.02	0.0012	0.0046	0.029
(PHED scenario 4 and 11): Wettable powders, open mixing/loading, and airblast application, open cab	N	0.35[1]	0.034	0.045	0.0046	0.43
	Y	0.016	0.02	0.045	0.0046	0.086
(PHED scenario 3 and 20): All liquids, open mixing/loading and low pressure handwand application (knapsack)	N	0.004	0.8[1]	0.00002	0.006	0.8
	Y	0.00004	0.4	0.00002	0.006	0.4
(PHED scenario 4 and 20): Wettable powders, open mixing/loading, and low pressure handwand application (knapsack)	N	0.0063[1]	0.8[1]	0.0008	0.006	0.81
	Y	0.0003	0.4	0.0008	0.006	0.41
(POEM scenario 5): All liquids, open mixing/loading and low pressure handwand application (vehicle mounted spray tank)	N	0.006	0.66	NA	0.009	0.68
	Y	0	0.32	NA	0.009	0.33
(PHED scenario 4 and POEM scenario 5) Wettable powder, open mixing/loading and low pressure handwand application (vehicle mounted spray tank)	N	0.032[1]	0.66	0.004	0.009	0.71
	Y	0.0014	0.32	0.004	0.009	0.33

*Exposure values for PHED were converted to systemic doses based on 70 kg person, 9% dermal absorption and 100% inhalation absorption. PHED estimates are for workers wearing a single layer of clothing (eg. long pants and long sleeved shirt).

NA: Not applicable (POEM does not estimate inhalational exposure during mixing/loading)

[1] Low confidence data; [2] No data for gloved portion of data in PHED, therefore extrapolated forward (90% reduction to hands), as per PHED Guidelines.

Table 53: MOE estimates for workers during mixing/loading and application of carbendazim products

Estimate	*Gloves*	**MOE***				
		Mixer/Loader Dermal	*Applicator Dermal*	*Mixer/Loader Inhalation*	*Applicator Inhalation*	*Total MOE#*
(PHED scenario 3): All liquids, open mixing/loading	N	**19**	NA	4167	NA	**19**
	Y	2500	NA	4167	NA	1500
(PHED scenario 4): Wettable powders, open mixing/loading	N	**14**	NA	111	NA	**12**
	Y	310	NA	111	NA	**82**
(PHED scenario 3 and 13): All liquids, open mixing/loading, and groundboom application, open cab	N	**25**	5000	5556	8333	**25**
	Y	3125	5000	5556	8333	1200
(PHED scenario 4 and 13): Wettable powder, open mixing/loading, and groundboom application, open cab	N	**19**	5000	146	8333	**17**
	Y	422	5000	146	8333	105
(PHED scenario 3 and 11): All liquids, open mixing/loading, and airblast application, open cab	N	**19**	147	5556	1087	**17**
	Y	2500	250	4167	1087	180
(PHED scenario 4 and 11): Wettable powder open mixing/loading, and airblast application, open cab	N	**14**	147	111	1087	**11**
	Y	319	250	111	1087	**59**
(PHED scenario 4 and 11): *As above, plus respiratory protection when mixing/loading*	*N*	***14***	*147*	*555*	*1087*	***13***
	Y	*319*	*250*	*555*	*1087*	*101*
(PHED scenario 3 and 20): All liquids, open mixing/loading and low pressure handwand application	N	1250	**6**	250000	833	**6**
	Y	125000	**13**	250000	833	**13**
(PHED scenario 4 and 20): Wettable powder, open mixing/loading, and low pressure handwand application	N	439	**6**	6093	833	**6**
	Y	792	**13**	6093	833	**13**
(POEM scenario 5): All liquids, open mixing/loading and low pressure handwand application (vehicle mounted spray tank)	N	833	**8**	NA	556	**8**
	Y	(no dermal exposure)	**16**	NA	556	**16**
(PHED scenario 4 and POEM scenario 5) Wettable powder, open mixing/loading and low pressure handwand application (vehicle mounted spray tank)	N	160	**8**	1300	556	**8**
	Y	3500	**16**	1300	556	**16**

***** Based on a NOEL of 5 mg/kg bw/day. # Calculated as: 1/Total MOE = 1/Mixer loader dermal MOE + 1/ Applicator Dermal MOE + 1/ Mixer/Loader Inhalation MOE + 1/ Applicator Inhalation MOE

Accidental oral ingestion

The new ARfD established for carbendazim in this review is quite low at 0.05 mg/kg bw. For a 70 kg worker, this equates to only 3.5 mg of active needing to be ingested to reach the ARfD. For the 500 g/L (SC) agricultural products, this equates to only 0.007 mL (7 µL) of the

concentrated product. For the 500 g/kg (WP) agricultural product this equates to 7 mg of product. For the timber preservative products at 75 g/L, 80 g/L and 100 g/L, this equates to around 0.04-0.05 mL of the concentrate. These small volumes make the likelihood of exceeding the ARfD by accidentally ingesting the concentrated product during handling sufficiently probable to be of concern, requiring the application of protective equipment.

The 500 g/L product is most commonly diluted 50 mL/100 L which is 2000-fold, making the amount to reach the ARfD 14 mL. Diluted 1000-fold (100 mL/100L) makes the amount 7 mL. For pasture, the dilution is around 350-fold, i.e. 2.5 mL. The most concentrated dilution is for chickpeas/faba beans/lentils at 500 mL/ha (min 100 L water/ha) which is a 200-fold dilution, i.e. 1.4 mL. It is more unlikely to ingest a sufficient volume during the application process, particularly when the product is handled in spray form.

Consequently, protection from ingestion is required during handling of the concentrated product and this can be afforded by a face shield for SC products and is covered by a respirator for the WP product.

Situation 1: Mixing and loading

Mixer/loader loader exposure when preparing up to 33 kg/day of carbendazim has been performed using PHED scenarios 3 and 4, based on "high confidence data" for Scenario 3 and "low and medium confidence data" for Scenario 4. The MOE estimates indicate that the extent of dermal exposure is acceptable with gloves for mixer/loader handling up to 33 kg/day carbendazim. Since exposure to carbendazim in this situation is not acceptable without gloves, workers should wear gloves when mixing and loading. Exposure by inhalation is at an acceptable level for SC products, with a MOE in excess of 4000. For the WP product, the total MOE is unacceptable, and inhalation exposure must be reduced using a respirator when handling the concentrated product.

Aside from repeat-dose effects evaluated in PHED, there are three acute hazards of concern. There is slight skin and eye irritation expected, as calculated in extrapolated data (Appendix III) which requires gloves and eye protection to be worn for handling undiluted product. There is also the issue of accidental acute oral ingestion, as the ARfD is quite low at 0.05 mg/kg bw/d equating to only 3.5 mg of carbendazim (see discussion above 'Accidental acute oral ingestion'). It is concluded that a face shield is required to protect from accidental ingestion during handling of the concentrated SC product and a full facepiece respirator when handling the WP product.

Therefore, workers are required to wear gloves and face shield when mixing and loading the SC products and gloves and a full facepiece respirator when mixing and loading the WP product.

Situation 2: Application by boom spray for field crops (open cab)

For most large-scale spray applications, operators would use vehicle mounted or drawn spray rigs adapted, where necessary, to direct the spray mixture between plant rows. In the absence of relevant exposure studies, applicator exposure was estimated using PHED scenario 13 (groundboom, open cab). Based on the MOE estimates, dermal exposure for a mixer/loader handling 25 kg/day of carbendazim is acceptable only with gloves, however dermal exposure during application is acceptable without gloves. Inhalation MOEs are acceptable during mixing/loading and application.

Conclusions: Mixing/loading carbendazim without gloves, results in unacceptable MOEs. In addition carbendazim SC 500 g/L products can cause slight skin irritation, as noted in extrapolated toxicology data. Therefore, workers should wear gloves when mixing/loading carbendazim. As discussed under Situation 1, a face shield or full facepiece respirator is required when preparing the spray. Based on PHED, no dermal PPE is required for workers applying carbendazim by groundboom spray apparatus from an open cab vehicle at the highest anticipated daily work rate of 25 kg/day. No inhalation PPE is required during application of carbendazim. Therefore, application of carbendazim by groundboom is supported.

Situation 3: Application by airblast for orchard crops (open cab)

In the absence of relevant exposure studies, applicator exposure was estimated using PHED scenario 11 (airblast, open cab). Based on the MOE estimates, dermal exposure for a mixer/loader handling 33 kg/day of carbendazim is acceptable with gloves, and for applicators is acceptable without gloves. For the SC products the total MOE is acceptable without respiratory protection. For the WP product, the total MOE is unacceptable (MOE = 59). Wearing a respirator during mixing/loading (80% reduction in inhalation exposure) brings the total MOE to 101. On this basis it is sufficient to wear a respirator during mixing/loading and no respiratory protection is required during application.

Conclusions: Mixing/loading carbendazim without gloves, results in unacceptable MOEs. In addition carbendazim SC 500 g/L products can cause slight skin and eye irritation, as noted in extrapolated toxicology data. Therefore, workers should wear gloves when mixing/loading carbendazim. As discussed under Situation 1, a face shield or full facepiece respirator is required when preparing the spray to prevent accidental oral ingestion and to protect against eye irritation. No dermal PPE is required for workers applying carbendazim by airblast spray apparatus from an open cab vehicle at the highest anticipated daily work rate of 33 kg/day. No inhalation PPE is required during application of carbendazim. Therefore, application of carbendazim by airblast is supported.

Situation 4: Application of SC formulations by hand-held equipment on ornamentals and turf

Carbendazim products are used for the control of fungal disease in ornamentals and turf, with a maximal application rate of 3 kg/ha. If hand-held equipment is used with a knapsack (15 L), it is unlikely that an area larger than 0.2 ha is treated daily which would involve handling 0.6 kg of carbendazim. If hand-held equipment is used with a vehicle-mounted spray tank (1000 L) mounted on a vehicle, it is unlikely that an area larger than 1 ha is treated daily which would involve handling 3 kg of carbendazim The exposure estimate is therefore be made on the assumption that 0.6 or 3 kg of carbendazim is applied.

Operators are likely to employ a low pressure handwand sprayer. The same person would normally undertake mixing/loading and the spraying operation. In the absence of exposure studies, the most relevant available method for estimating exposure is PHED scenario 20 for knapsack and POEM scenario 5 for vehicle-mounted spray tank. Based on the MOE estimates (Table 53), dermal exposure during application is unacceptable for both scenarios (13 and 16 with gloves for PHED and POEM, respectively). In addition, the unacceptable dermal dose cannot be mitigated with the addition of cotton overalls, assuming a 90% reduction in exposure. With overalls, the MOEs are still unacceptable (25 and 31, with gloves for PHED and POEM respectively). Inhalation MOEs are acceptable during mixing/loading (knapsack) and application (knapsack and vehicle-mounted).

Conclusions: Since the exposure to workers during the application of carbendazim to turf and ornamentals at the highest anticipated daily work rate of 0.6 kg (knapsack) or 3 kg (vehicle-mounted spray tank) results in unacceptable MOEs, despite the use of gloves and overalls, the use of carbendazim in these situations is not supported.

Situation 5: Application of SC formulations by dipping

Carbendazim products may be used post-harvest to treat various fruits by dipping. The produce are treated in a solution containing up to 0.2 L or 0.2 kg product/100 L water. It may be assumed that operators are most likely to lower a porous basket containing the materials into a dipping drum and then remove and dry the treated materials. Worker exposure when preparing the dipping solution would be similar to that occurred when mixing the products for spray application. Operators would probably not have to prepare more than 1000 L of dipping solution a day and so would not be exposed to more than 2 kg carbendazim.

There are no available studies or model suitable for estimating exposure when dipping and handling treated plant materials. However, from PHED scenario 3, the MOE is acceptable with gloves for a worker who mixes/loads up to 33 kg/day of carbendazim.

Conclusions: Mixing/loading carbendazim without gloves, results in unacceptable MOEs. In addition carbendazim SC 500 g/L products can cause slight skin and eye irritation, as noted in extrapolated toxicology data. Therefore, workers should wear gloves when mixing/loading carbendazim in this situation. As discussed under Situation 1, a face shield or full facepiece respirator is required when preparing the dip to prevent accidental oral ingestion and this will also protect against eye irritation. No PPE is required during dipping operations and when handling treated materials.

Overall Conclusion:

Operators mixing/loading SC 500 g/L and WP 500 g/kg carbendazim products should wear gloves and eye protection because carbendazim can cause slight skin and eye irritation. Face shields or full facepiece respirators are required due to the small volume which needs to be ingested to reach the ARfD. Operators applying carbendazim are likely to be exposed mainly via the dermal route. Even at the maximum anticipated daily work rates, spray operators applying carbendazim with airblast or groundboom equipment are not heavily exposed, and engineering controls are not required to protect them. It is not expected that when in the diluted form and being applied by spray, that sufficient quantities would be ingested to reach

the ARfD, hence face shields/respirators are not required during application. For the same reasons as above, gloves and face shields/respirators are required when preparing dipping solutions, but not during the dipping process. Therefore, the OCSEH has no objections to the continuation of these uses.

It is likely that operators applying carbendazim to ornamental plants and turf by hand-held equipment will be significantly exposed to the chemical and even with gloves and an additional layer (overalls), exposure remains unacceptable. Therefore, the OCSEH does not support the continuation of these uses.

4.2 Re-entry exposure

Workers re-entering treated crops, orchards or other areas, may be exposed to carbendazim, principally via the dermal route. The most probable source of exposure will be from making contact with carbendazim residues on foliage or fruit. In Table 54, re-entry intervals (REIs) have been calculated for food and non-food crops. The calculations assume that:

- The initial dislodgeable foliar residue (DFR) will be 5% of the application rate of carbendazim for turf and 20% for other crops. Where carbendazim may be applied repeatedly, further calculations have been performed to cover workers re-entering after subsequent treatments.

- Given that no residue studies have been submitted, the half-life of carbendazim transferable residues on turf and foliage has been estimated to be up to 6 months (or 180 days), based on the findings in the JMPR report (1995).

- According to the JMPR report (1995), carbendazim residues are expected to persist on leaf surfaces and to increase with each successive application. This is consistent with the long half-life and relative stability of the compound. Carbendazim only undergoes limited metabolism in the plant. The residues levels assessed by JMPR (1973) suggest that the decline is characteristic of growth dilution, more than any other means. To evaluate the build-up following subsequent treatments, the levels remaining on the day of re-application have been added to the new levels added with a subsequent treatment and reassessed. The results are in the table below. The calculations have been based on the shortest interval and the highest application rate, as specified on the product label.

- Dermal exposure (mg/kg bw) = (DFR level x TC x 8) ÷ bw where TC is the transfer coefficient (in cm^2/hr) and the workday is 8 hours. Bodyweight is 70 kg.

- Absorbed daily dose (mg/kg bw) = dermal exposure x dermal absorption factor (9%).

- MOE = NOEL (5 mg/kg bw/d) /Absorbed daily dose.

Table 54: Re-entry intervals for crops treated with carbendazim: following single application (appl) and reapplication.

Crop	Activity	Constants		Single application			Reapplication			
				Day 0 of first appl			Day 0 of repeat appl			
		TC (cm^2/h)	DFR ($\mu g/cm^2$)	Absorbed dose (mg/kg bw)	MOE	REI (days)	Absorbed dose remaining from previous appl at time of subsequent appl [2] (mg/kg bw)	Additive absorbed dose (mg/kg bw)	MOE	REI (days)
Cucurbits (repeated at 7-14 day intervals)	Irrigation, scouting, weeding	1000^	1.06	0.011	460	0	2nd appl: 0.01 (day 7)	0.02	250	0
Grapes [1]	Girdling, cutting, turning	10000^^	2.1	0.216	**23**	**MOE=46 at 180 days**	**Unacceptable following single application**			
Chickpeas, faba beans and lentils (maximum 2 treatments at 14-day interval)	Irrigation, scouting, weeding	1000^	0.96	0.0099	505	0	2nd appl: 0.009 (day 14)	0.019	263	0
Pasture and red clover (2 treatments at 30-day intervals. Possibly additional treatment at 30-day interval)	Irrigation, scouting, hand weeding, pruning, training, tying	1900^	1.5	0.029	172	0	2nd appl: 0.025 (day 30)	0.055	**90**	**28**
							3rd appl: 0.05 (day 30)	0.08	**63**	**120**
Roses (repeated at 7-14 day intervals, during the growing season)	Bundling, sorting, cutting	5000#	1.06	0.055	**92**	**23**	2nd appl: 0.053 (day 7)	0.1	**50**	**MOE=92 at 180 days**
							3rd appl: Unacceptable following second application			
Stone fruits, custard apples, apples and pears[1]	Irrigation, scouting, hand weeding, pruning, training	1900*	7.1	0.139	**36**	**MOE=72 at 180 days**	**Unacceptable following single application**			
Macadamia nuts (maximum 2 treatments, 21-d interval)	Irrigation, scouting, hand weeding, pruning, training	1900*	1	0.0195	256	0	2nd appl: 0.018 (day 21)	0.04	125	0
Strawberries (repeat at 7-14 d intervals)	Harvesting	1500^	1.06	0.016	313	0	2nd appl: 0.016 (day 7)	0.032	155	0
							3rd appl: 0.03 (day 7)	0.05	100	0
							4th appl: 0.05 (day 7)	0.07	**71**	**66**
Turf [1]	Hand weeding, transplanting	10000***	1.5	0.154	**32**	**MOE=64 @180 days**	**Unacceptable following single application**			

*Popendorf (1992); **Krieger (1992); *** Knaak (2000); ^US EPA default associated with low potential for post application dermal transfer; ^^ US EPA default associated with high potential for post application dermal transfer; #van Hemmen *et al* (2002) ; [1]Repeat applications are: Grapes (3 treatments at 3-month intervals. Possibly additional 2 treatments), stone fruit (up to 3 applications until petal fall), apples and pears (7-10 day intervals until petal fall), custard apples (max 4 sprays, minimum 14-d interval), turf (repeat at 4-wk intervals) - Not relevant here as single application is unacceptable.

[2]. The dissipation rate used in these calculations was 0.0038 which was estimated empirically based on a 180-day halflife.

Note: Other crops included on product labels are not included in this re-entry table as they are not treated in-field. Post-harvest uses are those for rockmelon, bananas and mangoes where the harvested fruit is submerged/dipped in the treatment. Pre-planting uses are for ginger seed pieces and sugar cane where pieces are submerged in the treatment.

Results:

Crops:

Crops with acceptable MOE's following a single or second application:

From the re-entry calculations shown in Table 53, it is evident that the following crops do not require a re-entry period following a single application: cucurbits, chickpeas/faba beans/lentils, pasture/red clover, macadamia nuts and strawberries. Of these, cucurbits, chickpeas/faba beans/lentils, and macadamia nuts are also acceptable following the second application.

Crops with unacceptable MOE's following repeat application(s):

Pasture and red clover has an MOE of 90 (REI of 28 days) following the second application (30 day interval) and an MOE of 63 (REI 120 days) following the third application. The exposure can be sufficiently reduced through the use of gloves to bring the MOE above 100 from day 1. Thus, gloves should be specified when entering pasture and red clover treated with products containing carbendazim. Strawberries are acceptable up until the fourth application with an MOE of 71 and REI of 66 days. Again, the exposure can be sufficiently reduced through the use of gloves, which should be specified for hand harvesting of strawberries treated with products containing carbendazim.

Crops with unacceptable MOE's following a single application:

From the table, it is evident that the following crops fail on re-entry after only a single application: grapes (MOE 23, REI>180 d) and stone fruits/ custard apples/apples/pears (MOE 36, REI >180 d). The labels specify re-application to all of these crops. Re-application would result in even lower MOEs, which can not be mitigated through the use of reasonable PPE (i.e. gloves). The application of more onerous PPE (e.g. overalls) is not considered appropriate given the long re-entry intervals (>180 days). For roses, although the first application results in a reasonable MOE (92) which could be mitigated with PPE, following a second application the MOE is unacceptably high for an extended period (MOE 50, REI >180 d).

Turf:

Greenkeepers may be exposed to carbendazim when re-entering treated areas to perform management activities such as mowing, weeding and transplanting. It is assumed that the initial DFR on turf is 5% of the application rate and a US EPA transfer coefficient (TC) of 10000 cm^2/hr is applied to cover these activities. At an application rate of 3 kg/ha re-entry is unacceptable after a single treatment (as shown in Table 54) with an MOE of 32 at Day 0 and an MOE of 65 at Day 180. The product labels specify repeat application to turf, which would result in even lower MOEs. The exposure cannot be mitigated with the use of reasonable PPE (i.e. gloves) and the use of more onerous PPE, such as overalls is not considered appropriate given the long re-entry intervals.

Conclusion:

Based on acceptable MOEs following single and subsequent applications, as recommended on the product label, use of carbendazim on cucurbits, chickpeas/faba beans/lentils, and macadamia nuts is supported from a re-entry perspective.

Based on unacceptable MOEs following single or subsequent applications, as specified on the product label, use on pasture/red clover and strawberries is supported, provided gloves are specified for re-entry.

Based on unacceptable MOEs for grapes, stone fruits, custard apples, apples, pears, turf and roses following a single application (or second application, for roses), and an extended re-entry interval (regardless of subsequent applications), the use of carbendazim on these crops is not supported.

4. ASSESSMENT OF EXPOSURE AND RISK –RESIDENTIAL/BYSTANDER

4.1 **Uses leading to residential/bystander exposure**

Carbendazim products can be used in on turf in public places such as golf courses, parks, sporting fields and bowling greens. They can also be used by commercial turf farmers in producing turf for sale to the public. Carbendazim products are not for use in the home garden, however, paint containing 0.5% carbendazim is available for domestic use[15].

4.2 Residential/bystander exposure calculations

Turf:
Methodology

Toddlers playing on treated turf may be exposed to carbendazim either through dermal contact followed by dermal absorption, or via hand-to-mouth exposure. Experience has shown that toddlers playing on turf treated with pesticides results in the highest exposure per kilogram bodyweight of any of the common residential/bystander scenarios.

The residential/bystander exposure to carbendazim active (g ai/day) has been calculated below.

Dermal dose:
The following formula has been used to estimate the dermally absorbed dose of carbendazim for a toddler playing on treated turf:

$$\mathbf{E_{ai,dermal} = AR \times TTR \times TC \times DA \times D(p)}$$

where, $\mathbf{E_{ai,dermal}}$ = Carbendazim exposure following dermal contact (g active/day)
AR = Application Rate (g active/m^2)
TTR = Turf Transferable Residues (unitless): The amount of pesticide available to be transferred after application
TC = Transfer Coefficient (m^2/hr): The area of treated turf contacted per hour of activity
DA = Dermal Absorption (unitless)
D(p) = Duration of play (hrs/day)

[15] Surface coatings (including paint but excluding antifouling paint) are not regulated by the APVMA.

Hand-to-mouth dose:
The following formula has been used to estimate the hand-to-mouth dose of carbendazim for a toddler playing on treated turf:

$$\mathbf{E_{ai,htm} = AR \times TTR \times SA \times FQ \times TE \times B \times D(p)}$$

where, $\mathbf{E_{ai,htm}}$ = Carbendazim exposure following hand-to-mouth exposure (g active/day)
AR = Application Rate (g active/m^2)
TTR = Transferable Residues (unitless): The amount of pesticide available to be transferred after application
SA = surface area of hands (m^2/event)
FQ = frequency of hand-to-mouth activity (event/hr)
TE = Transfer efficiency of the portion mouthed (unitless)
B = Oral Bioavailability (unitless)
D(p) = Duration of play (hr/day)

Total dose:
The total dose of carbendazim per kg bodyweight can then be calculated using:

$$E_{turf} = \frac{E_{ai,dermal} + E_{ai,htm}}{BW}$$

where, $\mathbf{E_{turf}}$ = total carbendazim exposure from turf (g active/kg bw/day)
$\mathbf{E_{ai,dermal}}$ = carbendazim exposure following dermal contact (g active/day)
$\mathbf{E_{ai,htm}}$ = carbendazim exposure following hand-to-mouth exposure (g active/day)
BW = bodyweight (kg bw)

Assumptions for Residential/Bystander Exposure

- **AR** (Application Rate): The maximum application rate of carbendazim products to turf was 60 mL/100 m^2, which is equivalent to 0.3 g active/m^2.

- **TTR** (Turf Transferable Residues): The default Turf Transferable Residue level is **5%** (USEPA default). Carbendazim is known to have a long half-life (6 months) therefore, in this risk assessment, it has been assumed that there will not be any significant degradation of the active on treated turf. However, turf is regularly cut, and either removed to a remote location, or left in place to degrade. On average, the active is applied at 4-weekly intervals and although there is unlikely to be regular cuts initially, once established the turf is likely to be cut more often than pesticides are applied. In this situation, there will not be any significant build up of carbendazim on the turf.

- **TC** (Transfer coefficient): The default value for toddlers playing on turf is 8700 cm^2/hr = **0.87 m^2/hr** (USEPA, 1997[16]).

[16] USEPA (1997) Draft Standard Operating Procedures (SOPs) for Residential Exposure Assessment. 68-W6-0030. December 19, 1997.

- **DA** (Dermal absorption): As calculated in this report, the dermal absorption factor is 9%.

- **D(p)** (Duration of play): **2 hr/day**. This value is based both on the amount of time spent playing on backyard turf (95th percentile: 2 hr per day), and the proportion of play which occurs on turf containing transferable carbendazim residues (upper-bound assumption: 100%). Toddlers may contact carbendazim-treated turf at ovals, fairways or similar areas, or at home with treated turf laid. Therefore it is possible that a toddler will have daily exposure to turf which has been treated with carbendazim. Whether carbendazim residues are present on this turf will depend on a number of factors, such as when the turf was last treated, when it was last cut, and the half-life on turf. As stated in the transferable residues section above, this risk assessment has assumed that there will not be any significant degradation of carbendazim on treated turf, and that there will not be any significant build up on the turf. On this basis, the estimate of 2 hr/day is sufficient to account for one application of carbendazim to turf every 4 weeks.

- **SA** (surface area of hands): Each hand-to-mouth event is estimated to equal one to three fingers or 6.7-20 cm^2 per event, with a mean of 15 cm^2 (**0.0015 m^2**). This is consistent with USEPA (2002[17]).

- **FQ** (mouthing frequency): The mean mouthing frequency for 3-6 year olds is **9.5 events/hr** (USEPA, 2008[18]).

- **TE** (Transfer efficiency of the portion mouthed): 100% default has been used in the absence of any data to the contrary. The use of 100% also makes allowance for a toddler continuing to mouth contaminated hands throughout the day, some time after exposure to treated turf.

- **B (**Oral Bioavailability): 100% has been used, as the relevant NOEL is based on an oral study, and therefore takes into account oral bioavailability.

- **BW** (Body weight): **15 kg bw** has been used for this estimate. The median weight for a 2-3 year old in Australia is 15.4 kg (ABS, 1995[19]).

Residential/Bystander Exposure Calculation

Dermal dose:

The following formula has been used to estimate the dermally absorbed dose of carbendazim for a toddler playing on treated turf:

$$E_{ai,dermal} = AR \times TTR \times TC \times DA \times D(p)$$

[17] USEPA (2002) Revised OP Cumulative Risk Assessment. June 10, 2002. Accessed online at http://www.epa.gov/oppsrrd1/cumulative/rra-op/index.htm

[18] USEPA (2008) Child-specific exposure factors handbook. EPA/600/R-06/096F. September 2008. www.epa.gov/ncea.

[19] ABS (1995). National Nutrition Survey: Nutrient Intakes and Physical Measurements. Australian Bureau of Statistics. Canberra.

$E_{ai,dermal}$ = Carbendazim exposure following dermal contact (g active/day)
AR = 500 g/L product at 60 mL product/100 m^2 = 0.3 g active/m^2
TTR = 5% = 0.05
TC = 8700 cm^2/hr = 0.87 m^2/hr
DA = 9 % = 0.09
D(p) = 2 hrs/day

$$E_{ai,dermal} = 0.3 \times 0.05 \times 0.87 \times 0.09 \times 2$$

$E_{ai,dermal}$ = 2.3 mg carbendazim/day

Hand-to-mouth dose:
The following formula has been used to estimate the hand-to-mouth dose of carbendazim for a toddler playing on treated turf:

$E_{ai,htm}$ =AR x TTR x SA x FQ x TE x B x D(p)

$E_{ai,htm}$ = Carbendazim exposure following hand-to-mouth exposure (g active/day)
AR = 500 g/L product at 60 mL product/100 m^2 = 0.3 g active/m^2
TTR = 5% = 0.05
SA = 15 cm^2/event = 0.0015 m^2/event
FQ = 9.5 events/hr
TE = 1
B = 1
D(p) = 2 hrs/day

$$E_{ai,htm} = 0.3 \times 0.05 \times 0.0015 \times 9.5 \times 1 \times 1 \times 2$$

$E_{ai,htm}$ = 0.17 mg carbendazim/day

Total dose:
The total dose of carbendazim per kg bodyweight can then be calculated using:

$$E_{turf} = \frac{E_{ai,dermal} + E_{ai,htm}}{BW}$$

E_{turf} = Total carbendazim exposure from turf (g/kg bw/day)
$E_{ai,dermal}$ = 0.07 g active/day
$E_{ai,htm}$ = 0.01 g active/day
BW = 15 kg bw

$$E_{turf} = \frac{2.3 + 0.17}{15}$$

E_{turf} = 0.17 mg carbendazim/kg bw/day

Based on these calculations, it is concluded that a toddler playing on treated turf will receive a dermal dose of 2.3 mg carbendazim/day and an oral dose of 0.17 mg carbendazim/day, a total of 2.47 mg carbendazim/day, or for a 15 kg toddler, 0.17 mg/kg bw/d.

5.2 Risk assessment- Residential/bystander

Turf:
Carbendazim products can be used on turf in public places such as parks, sporting fields, golf courses and bowling greens. They can also be used by commercial turf farmers in producing turf for sale to the public. Toddlers playing on treated turf may be exposed to carbendazim either through dermal contact followed by dermal absorption, or via hand-to-mouth exposure. Experience has shown that toddlers playing on turf treated with pesticides results in the highest exposure per kilogram bodyweight of any of the common residential/bystander scenarios.

There are two likely scenarios where children may be exposed to carbendazim through playing on treated turf, either intermittently in public spaces or for longer periods on turf laid at home. In the scenario of playing in public areas, it would be expected that toddlers would not be playing on the same recently treated field every day for extended periods, therefore an acute exposure assessment is most relevant. Section 5.1 concluded that the likely total dose of carbendazim absorbed by a toddler playing on treated turf 2-hr/day is 2.47 mg carbendazim/day, or for a 15 kg toddler this equates to 0.17 mg/kg bw/d. The ARfD of 0.05 mg/kg bw is based on LOEL of 50 mg/kg bw/day (equivalent to a NOEL of 5 mg/kg bw/d) producing irreversible reproductive effects in male rats and is relevant to children. Comparing the dose received from playing on treated turf to the ARfD, it is evident that acute exposure to this amount of carbendazim is unacceptable.

In toddlers playing on treated turf at home, it is expected that turf will be laid once, possibly recently treated, and the toddler will be playing on the turf daily (2-hours/day). The long half-life of carbendazim indicates it can remain in the treated grass for up to 6 months, although the amount available would expect to decrease with cutting of the grass. Therefore, the maximum period of exposure would be 6 months. In this scenario, a short-term exposure assessment is most relevant. Assuming the worse case scenario where the levels of carbendazim are high (eg treated just before being laid in the home garden), children may be exposed to the same dose calculated previously (0.17 mg/kg bw/d). The lowest NOEL seen in the toxicology package is that used for the ADI (2.5 mg/kg bw, 2-year dog), however toddlers are not expected to be exposed for such a long period nor continue to show the hand-to-mouth behaviour that places them most at risk. Therefore, the most relevant NOEL is the same as that used for acute exposure, and that used to establish the ARfD (namely a LOEL of 50 mg/kg bw/day equivalent to a NOEL of 5 mg/kg bw/d).

There is uncertainty regarding the levels of transferable residues remaining in the turf over a period of time, as although carbendazim has a long half-life, it is expected that watering/rain and cutting/growth of the grass would be expected to reduce the amount available. The OCSEH has not evaluated any data that allows a lower level to be used.

Conclusion:

The use of carbendazim on turf in public places, or on turf for sale to the public, is likely to result in unacceptable risk to the public, particularly to toddlers who are the most sensitive sub-group based on their hand-to-mouth behaviour. Therefore, the use of carbendazim products on public lawns including parks, golf courses, bowling greens and other sport-playing fields, as well as on commercial turf, is not supported.

6. SAFETY DIRECTIONS

Carbendazim

The current OHS exposure-based risk assessment has indicated that workers should wear gloves and eye protection when mixing/loading and preparing the SC 500 g/L carbendazim products for use. In addition, workers should wear face shields or full facepiece respirators when handling the concentrated product.

The current safety directions for Australian SC products containing 500 g/L carbendazim are shown below.

Existing Safety Directions for carbendazim products (SC 500 g/L)

SC 500 g/L or less greater than 80 g/L	
Codes	*Safety Directions*
160 162 164	May irritate eyes and skin
210 211	Avoid contact with eyes and skin
279 280 281 282 290 292 294	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves
351	Wash hands after use
360 361 366	After each days use, wash gloves and contaminated clothing

While the acute oral toxicity of carbendazim is low in rats (LD_{50}> 2000), it is a teratogen and affects male fertility for which effect a NOEL (via the oral route) of 5 mg/kg bw/d in rats has been established. The current Safety Directions for carbendazim SC products (500 g/L or less) were set with the aim of limiting operator exposure to carbendazim to the lowest reasonably achievable extent. Hence, the warning statements 'Very dangerous, particularly the concentrate' (100 101) and 'Poisonous if inhaled, absorbed by skin contact or swallowed (130 131 132 133) and 'Do not inhale vapour or spray mist' (220 222 223) are appropriate and should be included. The existing 160, 162, 164 statements are appropriate because slight skin and eye irritation are anticipated from the 500 g/L SC products as are the 360 361 366 after use directions. Statement 351 ('Wash hands after use') should be replaced with statement 350 ('After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water'). In addition, due to the concerns over the small volume of product that needs to be ingested to reach the ARfD, a face shield (296) is required when handling the concentrated product, to protect against accidental ingestion. A faceshield will also protect against eye irritation.

Application by hand-held equipment to ornamentals and turf is not supported, based both on unacceptable exposure during mixing/loading and application, and due to unacceptable re-

entry exposure. These use patterns are no longer supported, and therefore no additional safety directions are required. No PPE is required during application for other use patterns.

Amendments to existing entry for carbendazim products (SC 500 g/L)

Carbendazim SC 500 g/L or less greater than 80 g/L	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
160 162 164	May irritate the eyes and skin
210 211	Avoid contact with eyes and skin
220 222 223	Do not inhale vapour or spray mist
279 280 281 282 290 294c 296	When opening the container and preparing spray or dip, wear elbow-length chemical resistant gloves and face shield.
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

The current safety directions for the Australian WP product containing 500 g/kg carbendazim are shown below:

Existing Safety Directions for carbendazim product (WP 500 g/kg)

Carbendazim WP all strengths	
Codes	*Safety Directions*
210 211	Avoid contact with eyes and skin
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water

Similar to the SC 500 g/L products, the warning statements ‘Very dangerous, particularly the concentrate’ (100 101) and ‘Poisonous if inhaled, absorbed by skin contact or swallowed (130 131 132 133) and ‘do not inhale dust or spray mist’ (220 221 223) are appropriate and should be included. Dusts formed from the product may cause slight irritation to the eyes and the respiratory tract but it is unlikely to cause skin irritation. Statements 360, 366 after use direction are appropriate. In addition, due to the concerns over the small volume of product that needs to be ingested to reach the ARfD, a respirator is required when handling the concentrated product, to protect against accidental ingestion/inhalation.

Application by hand-held equipment to ornamentals is not supported, based both on unacceptable exposure during mixing/loading and application, and due to unacceptable re-entry exposure. These use patterns are no longer supported, and therefore no additional safety directions are required. No PPE is required during application for other use patterns.

Amendments to existing entry for carbendazim products (WP 500 g/kg)

Carbendazim WP 500 g/kg or less

Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
160 162 163	May irritate the eyes and nose and throat
210 211	Avoid contact with eyes and skin
220 221 223	Do not inhale dust or spray mist
279 280 281 282 290 294c 301 302	When opening the container and preparing spray or dip, wear elbow-length chemical resistant gloves and a full facepiece respirator with dust cartridge or cannister.
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 364 366	After each days use, wash gloves, respirator and contaminated clothing

Timber preservatives:

The current safety directions for Australian SC products containing 80 g/L carbendazim are shown below.

Existing Safety Directions for carbendazim product (SC 80 g/L)

Carbendazim SC 80 g/L or less with dodecylbenzene sulfonic acid 450 g/L or less and n-methyl-2-pyrrolidone 450 g/L or less	
Codes	*Safety Directions*
129 132 133	Harmful if inhaled or swallowed
161 164	Will irritate the skin
207 162	Will damage the eyes
220 222 223	Do not inhale vapour or spray mist
279 281 282 290 292 294a 299	When preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length butyl rubber gloves and face shield or goggles
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 365 366	After each days use, wash gloves, face shield or goggles and contaminated clothing

Similar to the SC 500 g/L products, the warning statements ‘Very dangerous, particularly the concentrate” (100 101) and ‘Poisonous if inhaled, absorbed by skin contact or swallowed (130 131 132 133) are appropriate and should replace statements 129 132 133 (‘Harmful if inhaled or swallowed’). Butyl rubber gloves should be replaced with chemical resistant gloves. The current entry requires face shield or goggles for eye protection for the severe eye irritation, however due to concerns over the small volume of product that needs to be ingested to reach the ARfD, a face shield (296) is required when handling the concentrated product, to protect against accidental ingestion. An estimation of the toxicity of this product is included in Appendix III. The product is expected to be a severe skin and eye irritant, requiring “Will damage the eyes and skin” and “Avoid contact with eyes and skin”. Other existing statements remain appropriate.

Amendments to existing Safety Directions for carbendazim product (SC 80 g/L)

Carbendazim SC 80 g/L or less with dodecylbenzene sulfonic acid 450 g/L or less and n-methyl-2-pyrrolidone 450 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 211	Will damage the eyes and skin
210 211	Avoid contact with the eyes and skin
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

The current safety directions for the Australian EC product containing 75 g/L carbendazim are shown in the Table below.

Existing Safety Directions for carbendazim product (EC 75 g/L)

Carbendazim LD 75 g/L or less with zinc naphthenate	
Codes	*Safety Directions*
161 162	Will irritate the eyes
210 211	Avoid contact with eyes and skin
219 223	Avoid inhaling spray mist
279 283 290 294 299	When using the product wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves and face shield or goggles
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 365	After each day's use, wash gloves, face shield or goggles

Similar to the SC 80 g/L products, the warning statements 'Very dangerous, particularly the concentrate" (100 101) and 'Poisonous if inhaled, absorbed by skin contact or swallowed (130 131 132 133) are appropriate and should replace statements 129 132 133 ('Harmful if inhaled or swallowed'). Statements "do not inhale vapour or spray mist' (220 222 223) are also appropriate and should be included. Given that the product is likely to be a severe skin and eye irritant, 207 211 statements ('Will damage the eyes and skin') are considered appropriate as are the 340 342 and 340 343 directions to wash eyes and skin if contact is made with the product are appropriate for the moderate skin and severe eye irritation that is anticipated from the 75 g/L SC products. The 350 361 365 366 after use directions are also appropriate. A face shield is required over goggles, as accidental ingestion is also of concern. PVC gloves should be replaced with chemical resistant gloves.

Amendments to existing entry for carbendazim products (EC 75 g/L)

Carbendazim EC 75 g/L or less with zinc naphthenate 90 g/L or less and n-methyl-2-pyrrolidone 370 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 211	Will damage the eyes and skin
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

OCSEH has previously recommended that the existing safety directions for the product Antiblu CC Concentrate containing 100 g/L carbendazim and 450 g/L chlorothalonil. On the basis of information on the toxicity and concentration of each of the constituents, this product would be likely to exhibit very low acute oral, dermal and inhalational toxicity, moderate skin irritation, severe eye irritation and to be a possible skin sensitiser. The current safety directions for Australian EC product containing 100 g/L carbendazim are shown below.

Existing Safety Directions for carbendazim product (SC 100 g/L)

Chlorothalonil SC 720 g/L or less	
Codes	*Safety Directions*
206 162 164	Attacks eyes and skin
210 211	Avoid contact with eyes and skin
220 222 223	Do not inhale spray mist
180 181	Repeated exposure may cause allergic disorders. Sensitive workers should use protective clothing.
279 281 282 290 292 294 297	When preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves and goggles and disposable (mist) face mask covering mouth and nose.
306 330 331 332 340 342 343	If clothing becomes contaminated with product or wet with spray, remove clothing immediately. If product on skin, immediately wash area with soap and water. If product in eyes, wash it out immediately with water.
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 363 366	After each days use, wash gloves, goggles and contaminated clothing.

Similar to the SC 500 g/L products, the warning statements "Very dangerous, particularly the concentrate" (100 101) and 'Poisonous if inhaled, absorbed by skin contact or swallowed (130 131 132 133) are appropriate and should replace statements 129 132 133 ('Harmful if inhaled or swallowed'). Butyl rubber gloves should be replaced with chemical resistant gloves and the option of goggles removed as the face shield is needed to protect against accidental ingestion also. Other existing statements remain appropriate.

Amended Safety Directions for carbendazim product (SC 100 g/L)

Chlorothalonil SC 720 g/L or less with carbendazim 100 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if inhaled, absorbed by skin contact or swallowed
161 164	Will irritate the skin
207 162	Will damage the eyes
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
180	Repeated exposure may cause allergic disorders.
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

7. CONCLUSIONS AND RECOMMENDATIONS

Carbendazim

h. The OCSEH recommends that the APVMA should be satisfied that persons involved in preparing and applying carbendazim products, according to the revised label directions (details below), will not suffer from adverse effects.

i. Based on the likelihood of toxicologically unacceptable levels of dermal and oral exposure to the public, uses of carbendazim on parks, golf courses, bowling greens and other sport-playing fields, as well as on commercial turf, should cease.

j. The following uses of carbendazim are supported with minor changes to the Safety Directions which appear on the label (details below):

- Application to field crops by boomspray.
- Application to orchard crops by airblast.
- Application to plant materials by dipping.
- Application to timber by spraying and dipping.

k. The following uses of carbendazim are no longer supported, from an occupational health and safety perspective:
- Application to ornamental plants and commercial turf by hand-held equipment.

l. The following uses of carbendazim are supported without changes to the current use pattern, based on re-entry risk assessment:
- Cucurbits.
- Chickpeas/faba beans/lentils.
- Macadamia nuts.

Re-entry statement:

For cucurbits, chickpeas, faba beans and lentils, and macadamia nuts, the following re-entry statement is recommended on the product label:

"Do not allow entry into treated areas until the spray has dried, unless wearing cotton overalls buttoned to the neck and wrist (or equivalent clothing) and chemical resistant gloves. Clothing must be laundered after each day's use."

m. The following uses of carbendazim are supported with PPE specified for re-entry procedures:
    - Pasture and red clover.
    - Strawberries.

Re-entry statement:

For pasture and red clover, and strawberries, the following re-entry statements are recommended on the product label:

"Do not allow entry into treated areas until the spray has dried, unless wearing cotton overalls buttoned to the neck and wrist (or equivalent clothing) and chemical resistant gloves. Clothing must be laundered after each day's use."

"Do not allow entry into treated areas after the spray has dried, unless wearing chemical resistant gloves."

n. The following uses of carbendazim are no longer supported, based on unacceptable exposure during re-entry and extended re-entry intervals:
    - Grapes.
    - Stone fruits, custard apples, apples and pears.
    - Roses.
    - Turf.

o. The following Safety Directions are recommended, which will be included in the FAISD Handbook, and which should be included on the product label:

Amendments to existing entries for carbendazim products

Amended Entry

Carbendazim SC 500 g/L or less greater than 80 g/L	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
160 162 164	May irritate the eyes and skin
210 211	Avoid contact with eyes and skin
220 222 223	Do not inhale vapour or spray mist
279 280 281 282 290 294c 296	When opening the container and preparing spray or dip, wear elbow-length chemical resistant gloves and face shield.
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

Amended Entry

Carbendazim WP 500 g/kg or less	
	Safety Directions
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
160 162 163	May irritate the eyes and nose and throat
210 211	Avoid contact with eyes and skin
220 221 223	Do not inhale dust or spray mist
279 280 281 282 290 294c 301 302	When opening the container and preparing spray or dip, wear elbow-length chemical resistant gloves and a full facepiece respirator with dust cartridge or cannister.
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 364 366	After each days use, wash gloves, respirator and contaminated clothing

Amended Entry

Carbendazim SC 80 g/L or less with dodecylbenzene sulfonic acid 450 g/L or less and n-methyl-2-pyrrolidone 450 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 211	Will damage the eyes and skin
210 211	Avoid contact with the eyes and skin
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

Amended Entry

Carbendazim EC 75 g/L or less with zinc naphthenate 90 g/L or less and n-methyl-2-pyrrolidone 370 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 211	Will damage the eyes and skin
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

Amended Entry

Chlorothalonil SC 720 g/L or less with carbendazim 100 g/L or less	
Codes	*Safety Directions*
100 101	Very dangerous, particularly the concentrate
130 131 132 133	Poisonous if inhaled, absorbed by skin contact or swallowed
161 164	Will irritate the skin
207 162	Will damage the eyes
220 222 223	Do not inhale vapour or spray mist
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
180	Repeated exposure may cause allergic disorders.
279 280 281 282 290 292 294c 296	When opening the container and preparing spray or dip and using the prepared spray or dip wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length chemical resistant gloves and face shield
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 362 366	After each days use, wash gloves, face shield and contaminated clothing

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APPENDIX I: Declarations of composition for the active carbendazim - not included in this document

- Confidential Commercial Information regarding the composition of the actives has been considered by the OCSEH. It has been removed prior to publication

APPENDIX II: Estimation of toxicity of Australian carbendazim products

Commercial in Confidence material has been removed prior to publication

Information regarding the toxicity of Australian registered carbendazim products was used by OCSEH for the purposes of reviewing the current First Aid Instructions and Safety Directions (FAISDs). The toxicity of these products was estimated following consideration of the individual toxicity profiles of all constituents. Toxicological data were obtained from internal OCSEH databases of active and non-active constituents, the Registry of Toxic Effects of Chemical Substances (RTECS), Material Safety Data Sheets (MSDS) and the International Program on Chemical Safety's (IPCS) INCHEM service.

APPENDIX III: List of Clinical Chemistry, Haematology & Urinalysis Parameters

Clinical Chemistry	Haematology	Urinalyses
albumin	clotting parameters (clotting	appearance
ALP (alkaline phosphatase)	time, prothrombin time)	specific gravity
bilirubin (total)	erythrocyte count	glucose
calcium	hematocrit (packed cell volume)	ketones
chloride	haemoglobin (Hb)	sediment (microscopic)
cholesterol (total)	leucocyte differential count	occult blood
creatinine (blood)	leucocyte total count	pH
gamma-glutamyl transpeptidase (GGT)	platelet count	protein
globulin	reticulocyte count	volume
glucose (blood)	MCH	bilirubin
LDH (serum lactate dehydrogenase)	MCHC	urobilinogen
phosphorus	MCV	reducing substances
potassium	blood smear	
protein (total)		
SGPT (serum alanine aminotransferase)		
SGOT (serum aspartate aminotransferase)		
sodium		
triglycerides		
urea nitrogen (blood)		
CPK (creatinine phosphokinase)		

APPENDIX IV: Organs for Weight Determination and Histopathological Examination

Organs Weighed	Tissues Examined		
Adrenals	Adrenals	heart	prostate
Brain	aorta	ileum	rectum
Gonads	blood smear	jejunum	salivary gland
Heart	bone	kidneys	seminal vesicle
Kidneys	bone marrow	lacrimal gland	skin
Liver	brain (3 levels)	liver	spinal cord (cervical
Spleen	caecum	lungs	thoracic, lumbar)
Thyroid	colon	lymph nodes	spleen
(w/parathyroid)	duodenum	mammary gland	sternum
	epididymes	muscle (smooth)	stomach
	eyes	muscle (skeletal)	testes
	eyes (optic nerve)	nerve (peripheral)	thymus
	gall bladder	oesophagus	thyroid
	Harderian glands	ovaries	(w/parathyroid)
	head - 3 sections	pancreas	trachea
	(nasal cavity, para-	pituitary	urinary bladder
	nasal sinus, tongue,		uterus
	oral cavity, naso-		vagina
	pharynx, inner-ear)		Zymbal's gland
			gross lesions

APPENDIX V: Reproductive and Developmental Indices

$$\textbf{Male/female mating index } (\%) = \frac{\text{number of males/females with confirmed mating*}}{\text{number of males/females placed with females/males}} \times 100$$

* defined by females with vaginal sperm or that gave birth to a litter or with pups/foetuses in utero

$$\textbf{Male fertility index (\%)} = \frac{\text{number of males proving their fertility*}}{\text{number of males placed with females/males}} \times 100$$

* defined by a female giving birth to a litter or with pups/foetuses in utero

$$\textbf{Female fertility index (\%)} = \frac{\text{number of females pregnant*}}{\text{number of females mated**}} \times 100$$

* defined as the number of females that gave birth to a litter or with pup/foetuses in utero

** defined as the number of females with vaginal sperm or that gave birth to a litter or with pups/foetuses in utero

$$\textbf{Gestation index (\%)} = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant*}} \times 100$$

* defined as the number of females that gave birth to a litter or with pups/foetuses in utero

$$\textbf{Live birth index (\%)} = \frac{\text{number of live born pups at birth}}{\text{total number of pups born}} \times 100$$

$$\textbf{Viability index (\%)} = \frac{\text{number of live pups on day 4* after birth}}{\text{number of live born pups on the day of birth}} \times 100$$

* before standardisation of litters (i.e. before culling)

$$\textbf{Lactation index (\%)} = \frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4* after birth}}.$$

* after standardisation of litters (i.e. after culling)

$$\textbf{Sex ratio} = \frac{\text{number of live male or female pups on day 0/21}}{\text{number of live male and female pups on day 0/21}} \times 100$$

$$\textbf{Conception rate (\%)} = \frac{\text{number of pregnant animals}}{\text{number of fertilised animals}} \times 100$$

$$\textbf{Preimplantation loss (\%)} = \frac{\text{number of corpora lutea} - \text{number of implantations}}{\text{number of corpora lutea}} \times 100$$

$$\textbf{Post implantation loss (\%)} = \frac{\text{number of implantations} - \text{number of live foetuses}}{\text{number of implantation}} \times 100$$

APPENDIX VI: Standard FOB parameters

Observations	Parameters
Home cage observations	Posture, piloerection, gait abnormalities, involuntary motor movements, vocalisations and any other abnormalities
Handling observations	Ease of removal from cage, reaction to being handled, muscle tone, palpebral closure, pupil size, pupil response, lacrimation, salivation, stains and any other abnormalities
Open field observations	Piloerection, respiratory abnormalities, posture, involuntary motor movements, stereotypy, bizarre behaviour, gait abnormalities, vocalisations, arousal, rearing, defecation, urination and any other abnormalities
Physiological observations	Catalepsy, body temperature, bodyweight