AUSTRALIAN PESTICIDES AND VETERINARY MEDICINES AUTHORITY

AUSTRALIA

CHEMICAL REVIEW PROGRAM

HUMAN HEALTH RISK ASSESSMENT

THIOPHANATE-METHYL

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 combined human health risk assessment covering both the public and occupational health issues associated with the use of thiophanate-methyl in Australia. The document is divided into two parts: Part I is an evaluation of the mammalian toxicology of thiophanate-methyl and is aimed at reviewing the existing public health standards to ensure that the continued use of thiophanate-methyl does not pose unacceptable risks to the general population. Part II of this document deals with the risks associated with the professional use of thiophanate-methyl.

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

Thiophanate-methyl is a broad-spectrum systemic fungicide with protective and curative action. At the commencement of this review there were three registered thiophanate-methyl products in Australia: two wettable powder formulations (one containing 150g/kg etridiazole and 250g/kg thiophanate-methyl and one containing 640g/kg mancozeb and 156g/kg thiophanate-methyl), and one granular formulation (containing 30g/kg etridiazole and 50g/kg thiophanate-methyl). These three products are registered for the control of soil-borne diseases of ornamental plants and are applied either directly to the soil (evenly mixed with the soil) or as a spray. Thiophanate-methyl products have not been marketed for home or garden use.

Thiophanate-methyl 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 or impair human fertility and consequent risks to workers using thiophanate-methyl products. In reviewing this concern, the OCSEH examined all of the available data and concluded that the continued use of thiophanate-methyl products in accordance with label instructions would not be likely to cause birth defects or impair human fertility in people exposed to it during its handling or people exposed to its residues.

The current Australian ADI for thiophanate-methyl is 0.02 mg/kg bw/day based on a NOEL of 2 mg/kg bw/d in a 2-year rat study. Following a review of submitted and archived data, a revised ADI of 0.08 mg/kg bw/d was established, based on effects in the thyroid associated with long-term exposure. No acute reference dose (ARfD) for thiophanate-methyl had been established. Following a review of submitted and archived data, an ARfD of 0.2 mg/kg bw has been established in this review by applying a safety factor of 100 to the NOEL of 20 mg/kg bw/d for foetal skeletal variations seen in a rabbit developmental study at 40 mg/kg bw/d. This review noted that the acute inhalational toxicity warranted inclusion into S6 of the SUSDP, with a cut-off to S5 at 25 per cent or less. This was agreed to by the NDPSC at the October 2009 Meeting. This review has also recommended a new health-based guideline value for thiophanate-methyl in Australian drinking water of 0.09 mg/L.

No changes to the approval status of thiophanate-methyl have been proposed in this review. There is no objection on public and occupational health grounds to the continued registration of all three existing thiophanate-methyl products. The review considered that the existing Safety Directions including PPE for the thiophanate-methyl products remain appropriate, other than a change in the type of gloves specified.

CONSOLIDATED RECOMMENDATIONS TO THE APVMA FOR THIOPHANATE-METHYL

1. Approval Status

No change is recommended to the approval status of thiophanate-methyl.

2. Product Registration

There is no objection on public and occupational health grounds to the continued registration of existing thiophanate-methyl products.

3. Acceptable Daily Intake

The present review established a revised ADI for thiophanate-methyl of 0.08 mg/kg bw/d, by applying a safety factor of 100 to the NOEL of 8 mg/kg bw/d derived from a 1-year dog study for effects on the thyroid.

4. Acute Reference Dose

A new ARfD of 0.2 mg/kg/bw for thiophanate-methyl has been established by applying a safety factor of 100 to the NOEL of 20 mg/kg bw/d from a rabbit developmental study for increased foetal skeletal variations (supernumerary ribs) following exposure of dams to 40 mg/kg bw/d.

5. Water Quality Guidelines

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

6. Poisons Schedule

It was recommended that thiophanate-methyl be included in Schedule 6 of the SUSDP, with a cut-off to S5 for preparations containing 25 per cent or less of thiophanate-methyl. The NPDSC agreed with this recommendation at its 57th meeting, on 20-21 October 2009.

7. First Aid Instructions and Warning Statements

The existing First Aid Instructions for thiophanate-methyl remain appropriate. No warning statements are required for thiophanate-methyl products.

8. Occupational Health and Safety Considerations

a. The OCSEH has concluded that persons involved in preparing and applying thiophanate-methyl-based products according to the directions on the label, are not likely to suffer from adverse effects.

b. The following uses of thiophanate-methyl are supported without change to the current conditions of application:

- Application to ornamental plants by mechanical tractor and hand-held equipment.
- Application to soil.

Re-entry statement

The following re-entry statement is recommended on the product label:

"*Do not allow entry into treated areas for 12 hours after treatment. When prior entry is necessary, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and chemical resistant gloves. Clothing must be laundered after each day's use."*

9. Safety Directions

The existing safety directions of the three registered thiophanate-methyl products remain appropriate, except that PVC gloves are to be replaced by chemical resistant gloves.

Amended Entry

Etridiazole WP 161 g/kg or less with thiophanate-methyl 270 g/kg or less	
Codes	*Safety Directions*
161 162 164	Will irritate eyes and skin
210 211	Avoid contact with eyes and skin
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately water
279 280 281 282 290 292 294c 297	When opening the container and preparing mix/drench and using the prepared mix/drench, wear cotton overalls buttoned to the neck and wrist and a washable hat and chemical resistant gloves and goggles.
351	Wash hands after use
360 361 363 366	After each days use, wash goggles, gloves and contaminated clothing
Etridiazole GR 34 g/kg or less with thiophanate-methyl 54 g/kg or less	
Codes	
161 162 164	Will irritate eyes and skin
210 211	Avoid contact with eyes and skin
279 280 283 290 292b 294c 306	When opening the container and using the product, wear cotton overalls buttoned to the neck and wrist, and chemical resistant gloves and a disposable dust mask
351	Wash hands after use
360 361 366	After each days use, wash gloves and contaminated clothing
Mancozeb WP 700 g/kg or less with thiophanate-methyl 200 g/kg or less when packed in sealed water soluble sachets (application by hand spray)	
Codes	*Safety Directions*
161 162	Will irritate the eyes
210 162	Avoid contact with eyes
279 282 290 292b 294c 298	When using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and elbow length chemical resistant gloves and impervious footwear
340 343	If product in eyes, wash it out immediately water
351	Wash hands after use
360 361 366	After each day's use, wash gloves and contaminated clothing

Mancozeb WP 700 g/kg or less with thiophanate-methyl 200 g/kg or less when packed in sealed water soluble sachets (application by mechanical sprayer)

Codes	*Safety Directions*
161 162	Will irritate the eyes
210 162	Avoid contact with eyes
279 282 290 292b 295	When using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow length chemical resistant gloves
340 343	If product in eyes, wash it out immediately water
351	Wash hands after use
360 361 366	After each day's use, wash gloves and contaminated clothing

Deleted Entries

~~Thiophanate methyl HG LD 1.5 g/L or less~~	
210 211	Avoid contact with eyes and skin.
219 223	Avoid inhaling spray mist.
351	Wash hands after use.
~~Thiophanate methyl WP 700 g/kg or less~~	
210 211	Avoid contact with eyes and skin.
220 221 223	Do not inhale dust or spray mist.

PART I: TOXICOLOGICAL & PUBLIC HEALTH ASSESSMENT OF THIOPHANATE-METHYL

TOXICOLOGY HAZARD PROFILE OF THIOPHANATE-METHYL

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of oral absorption	Rapid absorption in rats; 70% based on urinary excretion within 96 hours (low dose)
Distribution	Liver, kidney and thyroid
Potential for accumulation	No potential for accumulation
Rate and extent of excretion (rat)	Rapid at low doses, 91% excreted within 24 h; approximately 65% in urine and 26% in faeces
Metabolism	Predominantly metabolised (71-88%); major metabolites 4-OH-TM, Carbendazim and 5-HBC-S
Toxicologically significant compounds (animals, plants and environment)	Thiophanate-methyl and carbendazim

Acute toxicity

Rat oral LD_{50} (mg/kg bw)	> 5000
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)	1700 mg/m^3
Worst inhalation LC_{50} in other species	No data
Skin irritation	Not irritant
Eye irritation	Not irritant
Skin sensitization	Sensitising (Maximisation test) Not sensitising (Buhler test)

Short-term toxicity

Target/critical effect	Liver and thyroid: increased weights, changes in cholesterol and thyroxine levels, and histopathological abnormalities
Lowest relevant oral NOEL (mg/kg bw/d)	14 (13-week rat study)
Lowest relevant dermal NOEL (mg/kg bw/d)	300 (21-day rabbit study)
Lowest relevant inhalation NOEC (mg/m^3)	No data

Genotoxicity	Thiophanate-methyl has weak aneugenic potential

Long-term toxicity and carcinogenicity

Target/critical effect	Thyroid: decreased thyroxine levels, increased relative thyroid weight and incidence of hypertrophy and hyperplasia in follicular epithelium
Lowest relevant NOEL (mg/kg bw/d)	8 (1-year dog study)

Carcinogenicity	No evidence of carcinogenicity

Reproductive toxicity

Reproduction target/critical effect	Reduced pup weight at maternal toxic doses in rats
Lowest relevant reproductive NOEL (mg/kg bw/d)	35

Developmental toxicity

Developmental target/critical effect	Minor skeletal variations at maternal toxic doses in rabbits
Lowest relevant developmental NOEL (mg/kg bw/d)	20

Delayed neurotoxicity	No data
Immunotoxicity	No data
Dermal absorption	8%

Summary	NOEL (mg/kg bw/d)	Study	Safety factor
ADI (0.08 mg/kg bw) [Thyroid toxicity]	8	1-year dog study	100
ARfD (0.2 mg/kg bw) [Supernumerary ribs]	20	Rabbit developmental study	100

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

SUMMARY OF MAIN FINDINGS

Thiophanate-methyl, has been examined in relation to its potential as a mitotic spindle poison which can induce teratogenic effects and possibly testicular toxicity. In contrast to benomyl and carbendazim, thiophanate-methyl did not induce teratogenic effects in laboratory animals following gavage dosing at up to 1000 mg/kg bw/d. Thiophanate-methyl had only a low aneugenic potential in *vitro* and *in vivo* studies. There is no evidence that thiophanate-methyl causes carcinogenicity. The absence of testicular and teratogenic effects in reproductive and developmental studies following treatment of animals with thiophanate-methyl could be attributable to low metabolic conversion to carbendazim.

HAZARD ASSESSMENT

Reasons for the Review

In 2004, OCSEH reviewed benomyl which is the parent compound of carbendazim. Benomyl 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.

The toxicological database for thiophanate-methyl is extensive and consists of unpublished reports generated by industry, in addition to a number of published studies. Most studies complied with GLP and were undertaken according to the current OECD protocols. The database is considered adequate for the purpose of defining the hazard of thiophanate-methyl.

Toxicokinetics and Metabolism

Thiophanate-methyl was rapidly absorbed in rats after oral administration. The extent of absorption may be dose-dependent, decreasing with increasing dose. At the high dose level, approximately 50% of the dose was not absorbed and excreted unchanged in the faeces. The highest residual levels occurred in the liver, thyroid, and kidneys. The elimination of thiophanate-methyl was rapid, with more than 90% in the urine and faeces within 24 h of administration. There was a shift towards faecal elimination between the low and high doses and after repeated doses. There was no indication of potential bioaccumulation. There were several possible routes of metabolism and one of which was via carbendazim. The major urinary metabolite was 5-hydroxycarbendazim sulphate (5-HBC-S; 21-42%). The major faecal metabolites were 4-hydroxythiophanate-methyl (4-OH-TM; 6-10%), carbendazim (0.5-3%). Unchanged thiophanate-methyl accounted for approximately 21-24% and approximately 50% of the administered radiolabel in the faeces after repeated low and high doses, respectively (Tanoue, 1992). Metabolism of thiophanate-methyl in mice is similar to that in rats (Nabetani 1993).

Acute Toxicity

Thiophanate-methyl has low acute oral toxicity (LD_{50} > 5000 mg/kg bw) and dermal toxicity in rats (LD_{50} > 2000 mg/kg bw). The inhalational toxicity is moderate (LC_{50} 1900 mg/m^3 in rats, 4 hours). It is a not a skin or eye irritant in rabbits. Thiophanate-methyl is not a skin sensitiser in guinea pigs. In a 1-year study in dogs (Auletta 1992) transient tremors were noted 2-4 hours after dosing by capsule for the first 3 weeks of treatment.

The product Banrot® 400WP Broad Spectrum Fungicide for Ornamentals (150g/kg etridiazole and 250 g/kg thiophanate–methyl), has low acute oral (LD_{50} >5000 mg/kg bw, two 2 deaths), dermal (LD_{50} >2000 mg/kg bw, with no deaths) and inhalation (LC_{50} >4480 mg/m^3, with no deaths) toxicity in rats. Banrot® 400 WP Fungicide is a moderate skin and eye irritant in rabbits, but is not a skin sensitiser in guinea pigs. A 0.09% aqueous suspension of Banrot® 400 WP Fungicide was not an eye irritant in rabbits.

The product Zyban WSB Broad Spectrum Fungicide for Ornamental Plants which contains 640 g/kg mancozeb and 156 g/kg thiophanate-methyl, has low acute oral toxicity in rats (LD_{50} >5000 mg/kg bw, with no deaths); low dermal toxicity in rabbits (LD_{50} >2000 mg/kg bw, with no deaths) and low inhalation (LC_{50} >2427 mg/m^3, with no deaths) toxicity in rats. The product is a moderate eye but not a skin irritant in rabbits or a skin sensitiser in guinea pigs.

Dermal Absorption

Thiophanate-methyl is notable in that the proportion of the compound applied to the skin of rats and humans *in vitro* which is absorbed appears to be largely independent of dose (frequently an inverse relationship is noted). Absorption of thiophanate-methyl was very limited. *In vitro* studies with human skin membranes revealed that total dermal absorption after 8-hour exposure was 0.07%, 0.26% and 0.43% of the administered dose for applications of 51, 0.5 and 0.13 mg/cm^2, respectively (Walters 1993). Percutaneous absorption in human skin *in vitro* is around 33%, 21% and 24% of that seen in rats for the three preparations, respectively. Consequently, the values obtained from the rat *in vivo* dermal absorption study would be overly conservative if employed directly in human risk assessment and can be reduced by the ratio of the absorption rates in human and rat skin *in vitro*. Following a dermal exposure period in rats with thiophanate-methyl at 8 or 877 µg/cm^2, *in vivo* dermal absorption was 53% and 23% of the dose for the two concentrations, respectively (Walters 1981). Applying an average correction factor of 0.26 (0.33 + 0.21 + 0.24 = 0.25) gives a human dermal absorption factor of around 6% for the high dose and 13% for the low dose. A dermal absorption factor of 10, therefore, is considered appropriate for human risk assessment purposes.

Genotoxicity

Thiophanate-methyl was tested for genotoxicity in a series of *in vitro* and *in vivo* assays. Most *in vitro* genetic toxicology tests with thiophanate-methyl gave negative results. An *in vitro* test showed an increased frequency of micro-nucleated cultured human peripheral blood lymphocytes in the absence of S9 activation (Marshall 1997a). The frequency of micro-nucleated lymphocytes was higher than the historical control range but the effects were not dose-related. There was little evidence of similar effects in the presence of metabolic activation at a dose eight times higher suggesting that the cytogenetic effects seen in the absence of metabolic activation were neutralised by the liver post-mitochondria fraction (Marshall 1997a). The *in vivo* cytogenetic micronucleus test in mice by Barale *et al* (1993) reported that while a single oral dose of carbendazim at 500 mg/kg bw caused a substantial increase in the frequency of polyploid mouse bone marrow cells (17 polyploid cells per 800 cells), a single dose of thiophanate-methyl at 1000 mg/kg bw had little effect (1 polyploid cells per 600 cells). In another *in vivo* micronucleus test in mice, thiophanate-methyl (doses up to 2000 mg/kg bw) caused a small increase of micro-nucleated immature erythrocytes compared to control but values were not dosed-related and were within the historical control

range (Proudlock 1999). In contrast, carbendazim at a similar dose caused a substantial increase in the frequency of micro-nucleated immature erythrocytes. The values were four times higher than control and were well outside the historical range. When rats were treated at doses up to 5000 mg/kg bw, cytogenetic effects were not observed in the bone marrow and spermatogonial cells (Makita *et al* 1973). Collectively, these data indicated that thiophanate-methyl has a low aneugenic potential and thus is unlikely to induce cytogenetic effects *in vivo*. This is in agreement with the conclusion made in the 1998 JMPR and IPCS reports.

Short-term Toxicity

A six month dietary study in mice reported reduced erythrocyte counts and hematocrit values, and histopathological effects in the liver at doses of 250 mg/kg bw/d and above. A 13-week oral study in rats reported the most sensitive toxicological effects to be changes in total cholesterol and thyroxine levels, increased thyroid and liver weights and histopathological changes in the thyroid and liver. The NOEL for these effects was 14 mg/kg bw/d. A three-month oral study in dogs reported follicular cell hypertrophy in the thyroid at the lowest dose tested of 50 mg/kg bw/d. Additionally, relative liver and thyroid weights were increased and tri-iodothyronine (T3) levels and bodyweight parameters were decreased at doses of 200 mg/kg bw/d and above.

Long-term Toxicity and Carcinogenicity

Chronic feeding studies in mice, rats and dogs found no evidence that thiophanate-methyl was carcinogenic (Tompkin 1993; Takaori 1993; Hashimoto Y & Tsubura Y 1972; Auletta 1992). High incidence of benign liver adenoma was observed in both sexes of mice treated with thiophanate-methyl (Tompkin 1993). Liver adenomas are known to develop spontaneously in many strains of mice, at relatively high incidence without intentional exposure to chemicals. An historical control database compiled by the US National Toxicology Program indicates that the spontaneous rate for liver adenoma in male/female mice ranges from 4-60%/2-50%, respectively (Haseman *et al* 1998). The observed incidence of liver adenomas in the study by Tompkin (1993) was well within the historical control range (0-48% for males and 0-38% for females). Life-time studies in dogs and rats using were negative for liver adenomas at doses up 6000 ppm (Takaori 1993; Hashimoto Y & Tsubura Y 1972; Auletta 1992). The increased incidence of liver adenomas observed in mice, appears to be a species-related phenomenon.

Long-term dietary studies in rats and dogs showed the thyroid to be the target organ of toxicity.

A study in Sprague Dawley rats reported reduced bodyweight gain, histopathological effects in the thyroid (increased hypertrophy of the follicular epithelium and decreased colloidal substance) and effects on spermatogenesis at the highest dose tested of 640 ppm (30/34 mg/kg bw/d for M/F respectively). The NOEL for these effects was 8 mg/kg bw/d. The histopathological observations of effects on spermatogenesis are considered to be treatment related but quantitatively different from the testicular toxicity of carbendazim. This is due to the absence of strong aneugenicity and effects in reproductive or developmental studies most likely the result of low metabolic conversion to carbendazim. Thiophanate-methyl is hence not considered to present a hazard of impaired human fertility, and testicular toxicity is not considered to be a critical endpoint of toxicity. In a study in Fischer 344 rats, increased kidney, liver and thyroid organ weights, increased urine protein, anaemia and histopathological changes in thyroid, liver and kidney were reported at doses of 54 mg/kg

bw/d and above. The NOEL for these effects was 9 mg/kg bw/d. A treatment related effect on spermatogenesis was not reported in this rat study. However, degeneration and atrophy of the testes was observed in all groups (including controls) at similar incidences and severity, which may have masked a treatment related effect on spermatogenesis.

A 1-year study in dogs reported the most sensitive toxicological effects to be decreased levels of thyroxine, increased relative thyroid weight and incidence of hypertrophy and hyperplasia in the follicular epithelium of the thyroid at doses of 40 mg/kg bw/d and above. The NOEL for this effect was 8 mg/kg bw/d is the basis for the established ADI.

Reproductive and Developmental Toxicity

Multi-generation studies conducted in rats revealed no evidence that thiophanate-methyl caused reproductive toxicity (Müller 1993; Palmer 1972). There was limited evidence that thiophanate-methyl was teratogenic in rats or rabbits (Rodwel 1981; Tesh 1986). Skeletal variations (supernumerary ribs, reduced lumbar vertebrae) occurred in rabbit studies (York 1997b) at 40 mg/kg bw/d, but only at maternotoxic doses. In contrast, benomyl and carbendazim induced foetal head and eye malformations in rats at doses ranging from 30-90 mg/kg bw/d, and in all cases with no signs of maternal toxicity.

Normal embryonic development is characterised by rapid and coordinated cell replication. Thus, mitotic interference is a potential mechanism underlying chemically-induced developmental effects. It is proposed that carbendazim and benomyl bind to and induces conformational changes within brain tubulin (Russel *et al* 1992). Their effects on foetal development are believed to be related to their 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 numerical chromosomal aberrations (aneuploidy and polyploidy), 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. The absence of any teratogenic effects by thiophanate-methyl could be attributable to its low potential to induce aneuploidy and polyploidy in mice and rats *in vivo* (Barale *et al* 1993; Proudlock 1999; Makita *et al* 1973).

Carbendazim is generally considered to be the biologically active form of benomyl and there is strong evidence to indicate that carbendazim, rather than benomyl, is responsible for the testicular and developmental toxicity of benomyl. This proposition is supported by the following observations:

- Benomyl is rapidly metabolised *in vivo* via carbendazim with approximately 70% of benomyl is converted to carbendazim within an hour of a single oral gavage dose of 900 mg/kg bw in rats (Sherman *et al* 1975). Benomyl was also rapidly metabolised *in vivo* via carbendazim after repeated doses. In rats, after 10 consecutive daily treatments with benomyl at 125 mg/kg bw/d by oral gavage, benomyl levels in blood were negligible one hour following the last treatment (Culik 1981b);

- In developmental studies where rats were dosed via gavage with carbendazim alone, foetal malformations were observed at doses between 20–90 mg/kg bw/d, and in all cases with no signs of maternal toxicity (Alvarez 1987; Hofmann & Peh (1987b);

- Testicular toxicity was observed in rats following a single gavage dose of 50 mg/kg bw carbendazim alone (Nakai *et al* 1992; Matsuo *et al* 1999). 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 and increased frequencies of micronuclei in spermatids;

- When rats were treated with equimolar concentrations of benomyl or carbendazim, either intraperitoneally or by direct injection into the testis, no significant testicular damage was observed 2 hours after benomyl administration while 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 (Lim & Miller 1997).

In contrast to benomyl, approximately 56% and 21% of the parent compound thiophanate-methyl was excreted in the faeces of female rats following a single oral dose of 170 mg/kg bw/d and 14 consecutive daily treatments of a much lower dose of 14 mg/kg bw/d, respectively (Tanoue 1992). The conversion rate of thiophanate-methyl to carbendazim is not known, however, carbendazim was the main metabolite of benomyl while thiophanate-methyl can be converted to other metabolites as well as carbendazim. The results suggest that the absence of testicular toxicity and teratogenic effects in reproductive and developmental studies following thiophanate-methyl exposure may be due a low metabolic conversion to carbendazim.

International NOELs for Developmental Toxicity Studies

The JMPR and IPCS cite a NOEL of 2 mg/kg bw/d in a rabbit developmental toxicity study (Tesh 1986). In the study of Tesh (1986), rabbits were gavaged with thiophanate-methyl at doses of 0, 2, 6 or 20 mg/kg bw/d which resulted in reduced maternal bodyweight at 6 and 20 mg/kg bw/d during the first 2-8 days of treatment but recovered thereafter (5% and 9% lower than control, respectively). Food consumption at 6 and 20 mg/kg bw/d during this period was also lower than the control group (13% and 30% lower, respectively). Reduction in bodyweight and food consumption at 6 mg/kg bw/d was not considered to be treatment-related since the effects were marginal and were not observed in a more recent developmental rabbit study at 5 and 10 mg/kg bw/d *(*York 1997b*).* The study of Tesh (1986), also reported increased incidence of foetuses with 13 pairs of ribs and/or 27 presacral vertebrae at 6 and 20 mg/kg bw/d. The increased incidences observed at 6 and 20 mg/kg bw/d were not dose-related, were on borderline with the historical control incidences for New Zealand White rabbits (from 86 studies), were not observed at 20 mg/kg bw/d in a more recent developmental study in rabbits (York 1997b) and thus were not considered to be treatment-related. Therefore, a NOEL of 6 mg/kg bw/d for maternal toxicity was established for this study based on reduced bodyweight in the dams at 20 mg/kg bw/d.

In a study by York *et al* (1997b) which gavaged pregnant rabbits at doses of 0, 5, 10, 20 or 40 mg/kg bw/d, reduced bodyweight gains in dams was not observed at 10 mg/kg bw/d. No developmental effects were seen at 20 mg/kg bw/d. Considering these two studies (Tesh 1986; York 1997b), an overall NOEL for maternal toxicity of 10 mg/kg bw/d may be derived, in view of dose-selection differences between the two studies (see Table below).

Reference	NOEL	LOEL	Dose selection
	(mg/kg bw/d)		
Tesh (1986)	6	20	0, 2, 6, 20
York (1997b)	10	20	0, 5, 10, 20, 40

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 1.

Table 1: Studies using thiophanate-methyl relevant for the establishment of an ADI and ARfD

Species (study type)	NOEL (mg/kg bw)	LOEL (mg/kg bw)	Toxicological Endpoint	Reference
Single dose studies/acute effects				
Dog (1-year, capsule)	40	200	Tremors seen 2-4 h after dosing, first 3 wks of treatment only	Auletta (1992)
Subchronic studies				
Mouse (6 months, diet)	50	231	Swollen, vacuolated hepatic cells	Noguchi (1970)
Rat (90 days, diet)	14	155	Increased liver and thyroid weight; increased incidence of hepatocellular swelling and follicular hyperplasia/hypertrophy in thyroid	Nishibe (1990)
Dog (90 days, capsule)	-	50	Follicular cell hypertrophy in thyroid	Auletta (1991)
Chronic studies				
CD-1 mice (78 weeks, diet)	29	123	Benign hepatocellular adenoma	Tompkin (1993)
Rat (2 years, diet)	9	54	Increased liver, kidney and thyroid weight, increased incidence of hepatocellular hypertrophy, follicular hyperplasia/hypertrophy in thyroid, nephropathy and anaemia	Takaori (1993)
Rat (2 years, diet)	8	30	Increased follicular hyperplasia/hypertrophy in thyroid and histopathological observations of effects on spermatogenesis	Hashimoto Y & Tsubura Y (1972)
Dog (1-year, capsule)	8	40	Increased thyroid weight, follicular hyperplasia/hypertrophy in thyroid and decreased thyroxine levels	Auletta (1992)
Reproduction studies				

Rat (2-generation reproduction, diet)	Dams and foetuses: 35	Dams and foetuses: 114	Decreased bodyweight gain and increased liver and thyroid weights (maternal) and decreased pup bodyweight	Müller (1993)
Developmental studies				
Rat (pilot developmental, gavage)	300 (dams) 1000 (foetuses)	1000 (dams) - (foetuses)	Reduced maternal bodyweight gain (dams);	Rodwel (1981a)
Rat (developmental, gavage)	300 (dams) 1000 (foetuses)	1000 (dams)	Reduced maternal bodyweight gain (dams)	Rodwel (1981b)
Rabbit (developmental, gavage)	6 (dams) 20 (foetuses)	20 (dams) - (foetuses	Reduced maternal bodyweight gains	Tesh (1986)
Rabbit (developmental, gavage)	10 (dams) 20 (foetuses)	20 (dams) 40 (foetuses)	Reduced maternal bodyweight gains; Increased incidence of skeletal variations (foetal)	York (1997b)

PUBLIC EXPOSURE ASSESSMENT

Residues in Food and Drinking Water

In Australia, the three thiophanate-methyl products are registered for the control of fungal diseases in ornamentals plants. Based on its current pattern of use, exposure of the general population to thiophanate-methyl residues in food commodities and in drinking water is considered toxicologically insignificant.

HUMAN RISK ASSESSMENT

Dietary risk assessment

The dietary risk assessment for thiophanate-methyl has been performed by the APVMA and FSANZ.

Re-entry risk assessment

The risk assessment for re-entry of the general public onto treated areas is covered in the OHS review of this report (See Part II).

CONSIDERATION OF PUBLIC HEALTH STANDARDS

Approval Status

There is no objection on toxicological grounds to the ongoing approval of thiophanate-methyl active constituent sourced from Mitsui & Co Australia Ltd.

Impurity Limits

From the declaration of composition from the manufacturer Mitsui & Co Australia Ltd, the active constituent thiophanate-methyl contains no impurities of toxicological concern.

Acceptable Daily Intake (ADI)

The Australian ADI for thiophanate-methyl in place at the commencement of this review was 0.02 mg/kg bw/d established in 1991. The ADI was derived by applying a safety factor of 100 to the NOEL of 2 mg/kg bw/d in a 2-year rat study in which increased incidences of degeneration and atrophy in testes at higher doses (Hashimoto & Tsubura 1972). In the study of Hashimoto and Tsubura (1972), rats were treated with thiophanate-methyl in the diet at doses of 0, 10, 40, 160 or 640 ppm (0, 0.5/0.5, 2/2, 8/8 and 30/34 mg/kg bw/d for M/F, respectively. Treatment related histopathological findings were observed only in the 640 ppm group. In males, an increase in hypertrophy of follicular epithelium and a decrease of colloidal substance in thyroid were observed. Histopathological observations of effects on spermatogenesis were noted in six males at 640 ppm, two males at 160 ppm and one each in the other groups including the control. The effects on spermatogenesis in one animal in each of the 10 and 40 ppm, and two animals in the 160 ppm dose groups are considered to be sporadic and not treatment related. This is consistent with hypospermatogenesis observed in one of the control animals. However, the effects on spermatogenesis in the 640 ppm dose group (6/35 animals) are considered to be treatment related. This is considered an appropriate conservative measure as macroscopic observations of testicular atrophy were observed in three of the six animals and histopathological observations of effects on spermatogenesis have been obtained in rats treated with the ethyl derivative of thiophanate at a similar dose (31.25 mg/kg bw/d). Additionally, a critical endpoint of toxicity for the metabolite carbendazim is testicular effects. It was concluded that the NOEL in this study was 8 mg/kg bw/d based on reduced bodyweight gains and histopathological changes in the thyroid and testes at the highest dose.

Inspection of the table of studies relevant for the establishment of an ADI reveals that the most sensitive toxicological end points in mice, rats and dogs were liver, thyroid and testes toxicity and the NOEL for these effects should be the basis for the establishment of the ADI. The lowest NOEL was 8 mg/kg bw/d in a 1-year study in dogs based on thyroid toxicity (Auletta, 1992). This NOEL and endpoint are supported by a 2-year rat study with the same NOEL based on thyroid toxicity (Hashimoto & Tsubura 1972). Since the database for thiophanate-methyl is adequate for characterising its toxicological profile, a 100-fold safety factor is appropriate. On this basis, a new ADI of 0.08 mg/kg bw/d for thiophanate-methyl was established based on thyroid toxicity in a 1-year dog study. This ADI is considered to be protective of effects on spermatogenesis observed in the 2-year rat study (Hashimoto & Tsubura 1972).

Acute Reference Dose (ARfD)

An Australian ARfD has not been previously established for thiophanate-methyl.

At doses of up to 2000 mg/kg bw thiophanate-methyl, no clinical signs were observed in a micronucleus test in mice (Proudlock 1999). There were no clinical signs observed in an acute oral toxicity study in rats following a single dose of 5000 mg/kg bw of thiophanate-methyl (Nishibe 1990a) or in rat developmental toxicity studies.

At doses of 200 mg/kg bw/d in a 1-year dog study (by capsule), transient tremors were seen 2-4 h after treatment in 7 out of 8 dogs for the first three weeks of the study (Auletta 1992). Although these effects were not noted in a 3-month dog study (by capsule) at doses up to 800 mg/kg bw/d (Auletta 1991), the tremors seen in the 1-year study are considered toxicologically relevant. No acute effects were noted at the next lowest dose of 40 mg/kg bw/d. As the tremors occurred shortly after dosing, they are considered an acute effect and considered to be an appropriate endpoint to establish an ARfD. If a 100-fold safety factor was used, an ARfD of 0.4 mg/kg bw would be established for thiophanate-methyl.

However, in a rabbit developmental study, there was an increase in foetal skeletal variations (supernumerary ribs) at 40 mg/kg bw/d (York 1997b). Although these variations occurred only in conjunction with maternotoxicity, it is possible that they were related to a single exposure. Therefore, to be protective of developmental effects which can occur following exposure *in utero*, a NOEL of 20 mg/kg bw/d from this rabbit developmental study is used to establish the ARfD. A 100-fold safety factor is used, incorporating 10-fold each for intra and interspecies variation. On this basis, an ARfD of 0.2 mg/kg bw is established for thiophanate-methyl. This ARfD is also protective of the tremors seen in the dog study.

Water Quality Guidelines

A health-based guideline value for thiophanate-methyl in drinking water has not been previously established.

Given that thiophanate-methyl rapidly converts to carbendazim and 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.

Poisons Scheduling

At the commencement of this review, thiophanate-methyl was not listed in the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP), based on its low toxicity. The following points should be noted in relation to the poison schedule of thiophanate-methyl:

- Thiophanate-methyl has low acute oral toxicity (LD_{50} > 5000 mg/kg bw) and dermal toxicity in rats (LD_{50} > 2000 mg/kg bw). The inhalational toxicity is moderate (LC_{50} 1900 mg/m^3 in rats, 4 hours). It is a not a skin or eye irritant. Thiophanate-methyl is not a skin sensitiser in guinea pigs;

- In repeat-dose studies in mice, rats and dogs, thiophanate-methyl caused liver and thyroid toxicity in mice, rats and dogs. The ADI is 0.08 mg/kg bw/d based on a NOEL of 8 mg/kg bw/d in a 1-year study in dogs and using a 100 fold safety factor;

- Thiophanate-methyl is not a reproductive toxicant in rats. The NOEL was 20 mg/kg bw/d for both adults and pups;

- Thiophanate-methyl is not a developmental toxicant in rats at doses of up to 1000 mg/kg bw/d. In rabbits, an increased incidence of foetal skeletal variations was seen at the dose that caused maternal toxicity (40 mg/kg bw/d);

- Thiophanate-methyl was only weakly aneugenic and does not show evidence of carcinogenicity.

- Thiophanate-methyl demonstrates effects on spermatogenesis in rats following long-term exposure at a dose of 640 ppm (30 mg/kg bw/d). However, this testicular toxicity is apparently quantitatively different from that of carbendazim. At comparatively moderate doses and following shorter term exposure, 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. The effects on the male reproductive system and foetal development probably arise from the interaction of carbendazim with tubulin, disrupting microtubule assembly and causing interference with cellular division and differentiation in somatic and germ cells, which has been demonstrated in cultured cells at physiologically relevant concentrations of carbendazim. Inhibition of tubulin results in numerical chromosomal aberrations (aneuploidy and polyploidy) and alterations in cell division rate and/or cell death. Thiophanate-methyl does not demonstrate any teratogenic or reproductive effect which could be associated with its low potential to induce aneuploidy and polyploidy in mice and rats *in vivo* (Barale *et al* 1993; Proudlock 1999; Makita *et al* 1973). This is possibly due to a low metabolic conversion to carbendazim. Thiophanate-methyl products are hence not considered to present a developmental or reproductive hazard to users and therefore a warning statement on products is not warranted.

The toxicological information for thiophanate-methyl suggests that thiophanate-methyl be placed in Schedule 6 based on its moderate inhalation toxicity. At its 57th meeting, on 20-21

October 2009, the NDPSC agreed with this recommendation, including a cut-off to Schedule 5 at 25 per cent thiophanate-methyl (NDPSC 2009[1])

First-Aid Instructions

No first aid instructions for thiophanate-methyl have been established. Based on the acute hazard of thiophanate-methyl, the following first aid directions are recommended.

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

Safety Directions

At the commencement of this review there were three registered thiophanate-methyl products in Australia: two wettable powder formulations (one containing 150g/kg etridiazole and 250g/kg thiophanate-methyl and one containing 640g/kg mancozeb and 156g/kg thiophanate-methyl), and one granular formulation (containing 30g/kg etridiazole and 50g/kg thiophanate-methyl). These three products are registered for the control of soil-borne diseases of ornamental plants and are applied either directly to the soil (evenly mixed with the soil) or as a spray. Thiophanate-methyl products have not been marketed for home or garden use. Public and occupational health assessments for these three products were conducted by the OCSEH in 2000 and 2001. The safety directions for the three Australian products containing thiophanate-methyl 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 thiophanate-methyl.

2. Product Registration

There is no objection on public health and occupational health and safety grounds to the continued registration of existing thiophanate-methyl products.

3. Acceptable Daily Intake

The present review established a new ADI for thiophanate-methyl of 0.08 mg/kg bw/d, based on a NOEL of 8 mg/kg bw/d in a 1-year dog study using a 100-fold safety factor.

4. Acute Reference Dose

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

A new ARfD of 0.2 mg/kg/bw for thiophanate-methyl has been established by applying a safety factor of 100 to the NOEL of 20 mg/kg bw/d from a rabbit developmental study for increased foetal skeletal variations (supernumerary ribs) following exposure of dams to 40 mg/kg bw/d.

5. Water Quality Guidelines

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

6. Poisons Schedule

It was recommended that thiophanate-methyl be included in Schedule 6 based on moderate inhalation toxicity, with a cut-off to Schedule 5 at 25 per cent or less. At its 57th meeting, on 20-21 October 2009, the NDPSC agreed wth this recommendation.

7. First Aid Instructions, Warning Statements and Safety Directions

Based on the acute hazard of thiophanate-methyl, the following first aid directions are recommended:

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

No warning statement is required for thiophanate-methyl products. The existing safety directions of the three registered thiophanate-methyl products remain appropriate, except that PVC gloves are to be replaced by chemical resistant gloves. In addition, there are two entries in the FAISD for which there are no registered products. These entries will be deleted.

Amended Entries

Etridiazole WP 161 g/kg or less with thiophanate-methyl 270 g/kg or less	
Codes	*Safety Directions*
161 162 164	Will irritate eyes and skin
210 211	Avoid contact with eyes and skin
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately water
279 280 281 282 290 292 294c 297	When opening the container and preparing mix/drench and using the prepared mix/drench, wear cotton overalls buttoned to the neck and wrist and a washable hat and chemical resistant gloves and goggles
351	Wash hands after use
360 361 363 366	After each days use, wash goggles, gloves and contaminated clothing
Etridiazole GR 34 g/kg or less with thiophanate-methyl 54 g/kg or less	
Codes	
161 162 164	Will irritate eyes and skin
210 211	Avoid contact with eyes and skin
279 280 283 290 292b 294c 306	When opening the container and using the product, wear cotton overalls buttoned to the neck and wrist, and chemical resistant gloves and a disposable dust mask
351	Wash hands after use
360 361 366	After each days use, wash gloves and contaminated clothing

Mancozeb WP 700 g/kg or less with thiophanate-methyl 200 g/kg or less when packed in sealed water soluble sachets (application by hand spray)	
Codes	*Safety Directions*
161 162	Will irritate the eyes
210 162	Avoid contact with eyes
279 282 290 292b 294c 298	When using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and elbow length chemical resistant gloves and impervious footwear
340 343	If product in eyes, wash it out immediately water
351	Wash hands after use
360 361 366	After each day's use, wash gloves and contaminated clothing
Mancozeb WP 700 g/kg or less with thiophanate-methyl 200 g/kg or less when packed in sealed water soluble sachets (application by mechanical sprayer)	
Codes	*Safety Directions*
161 162	Will irritate the eyes
210 162	Avoid contact with eyes
279 282 290 292b 295	When using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow length chemical resistant gloves
340 343	If product in eyes, wash it out immediately water
351	Wash hands after use
360 361 366	After each day's use, wash gloves and contaminated clothing

Deleted Entries

~~**Thiophanate methyl HG LD 1.5 g/L or less**~~	
210 211	Avoid contact with eyes and skin.
219 223	Avoid inhaling spray mist.
351	Wash hands after use.
~~**Thiophanate methyl WP 700 g/kg or less**~~	
210 211	Avoid contact with eyes and skin.
220 221 223	Do not inhale dust or spray mist.
351	Wash hands after use.

8. Occupational Health and Safety Considerations

a. The OCSEH recommends that the APVMA should be satisfied that persons involved in preparing and applying thiophanate-methyl-based products, will not suffer from adverse effects.

b. The following uses of thiophanate-methyl are supported without change to the current conditions of application:

- Application to ornamental plants by mechanical tractor and hand-held equipments.
- Application to soil.

c. A re-entry interval of 12 hours is recommended for persons performing management activities and persons should wear chemical resistant gloves and overalls if prior entry is required.

MAIN TOXICOLOGY REPORT

1. INTRODUCTION

Carbendazim is the primary metabolite of thiophanate-methyl and benomyl. The latter was reviewed by OCSEH in 2004. Thiophanate-methyl rapidly converts to carbendazim and was nominated for review based on concerns over the 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).

1.1 Public Health Considerations of Thiophanate-methyl in Australia

A limited amount of data was evaluated by the OCSEH or predecessors in the early 1990s.

ADI

At the commencement of the review the Australian ADI for thiophanate-methyl was 0.02 mg/kg bw/d, established in 1991. The ADI was based on a NOEL of 2 mg/kg bw/d in a 2-year rat study and a safety factor of 100 (Hashimoto 1972).

ARfD

At the commencement of the review no acute reference dose (ARfD) had been established for thiophanate-methyl.

Poisons Scheduling

Thiophanate-methyl was included in Appendix B (exempt list) up until the deletion of the Appendix in 1995. Considering re-instatement of the Appendix in 2002, the NDPSC agreed that the status of chemicals previously included on the list would remain unchanged (i.e. unscheduled), it was also agreed that that they should be reviewed at a later stage. Following re-instatement of this Appendix in 2003, thiophanate-methyl was no longer included in the list.

Drinking Water Guidelines

At the commencement of this review no guideline value or health value had been established for thiophanate-methyl in drinking water.

1.2 International Toxicology Assessments

US EPA

In October 2005 the US EPA published a RED for thiophanate-methyl and its main metabolite carbendazim. It was determined that the acute and chronic dietary risk from food and water treated with thiophanate-methyl was considered to be low. An acute RfD for the general

population of 0.4 mg/kg bw/day was set, based on a NOAEL of 40 mg/kg/day from a 1-year dog study where there was tremors 2-4 hours post-dosing in 7/8 dogs at 200 mg/kg bw/day. The USEPA added a 'Food Quality Protection Act (FQPA)' SF of 3, taking the value to 0.13 mg/kg/day. This additional safety factor is intended to protect for the special sensitivity in infants and children, or to compensate for an incomplete database. For females (13-50 y), the USEPA set an acute RfD of 0.2 mg/kg bw/d, based on a NOAEL of 20 mg/kg bw/d for supernumerary ribs in foetuses and decreased foetal weight of exposed dams in a rabbit developmental toxicity study and using a 100-fold uncertainty factor (analogous to a safety factor). The USEPA added a 'FQPA' SF of 3, taking the value to 0.067 mg/kg/day. A chronic RfD (analogous to an ADI) was set at 0.08 mg/kg bw/d, based on a NOAEL of 8 mg/kg bw/d for liver and thyroid toxicity in a 1-year dog study and using a 100-fold uncertainty factor. Their FQPA SF was 3, taking the value to 0.027 mg/kg/day.

The Cancer Assessment Review Committee has classified thiophanate-methyl as 'likely to be carcinogenic to humans' based on hepatocellular tumours in mice.

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

Thiophanate-methyl was evaluated toxicologically by the Joint Meeting in 1973, 1975, 1977, 1995, 1998 and 2006. An ADI of 0-0.08 mg/kg bw was allocated in 1973, on the basis of a NOAEL of 8 mg/kg bw/d in a three-generation study of reproductive toxicity in rats and a safety factor of 100. This ADI was confirmed in 1975 and 1977. Additional data that became available and were reviewed at the 1995 Meeting, at which time an ADI of 0-0.02 mg/kg bw/d was established on the basis of a NOAEL of 2 mg/kg bw/d in a study of developmental toxicity in rabbits for reduced bodyweight in the dams at 20 mg/kg bw/d.

New information on the metabolism of thiophanate-methyl and the results of a second study of developmental toxicity in rabbits were reviewed at the 1998 Meeting. An ADI of 0-0.08 mg/kg bw/d was established on the basis of the NOAEL of 8 mg/kg bw per day in a three-generation study of reproductive toxicity in rats and in a one-year study in dogs, both of which were evaluated at earlier meetings, and a safety factor of 100. An ARfD was not required because thiophanate-methyl is of low acute toxicity when administered orally or dermally and is only slightly toxic when administered by inhalation. The Meeting concluded that thiophanate-methyl was not mutagenic, but had weak aneugenic potential. In 2006 JMPR conducted an evaluation for an ARfD for thiophanate-methyl. Acute effects seen in the 1-year dog study at 200 mg/kg bw/d, used by other International Agencies to establish an acute RfD for the general population, were discounted as not relevant as they were not seen in a 3-month dog study at 800 mg/kg bw/d. The developmental effects observed in rabbits at 40 mg/kg bw/d were not considered to be elicited by a single exposure. It was concluded that an ARfD was not necessary for thiophanate-methyl in view of its low acute toxicity, the absence of relevant developmental toxicity that could be a consequence of acute exposure, the absence of relevant findings in a study of acute neurotoxicity, and the absence of any other toxicological effect that would be likely to be elicited by a single dose.

Canadian Pest Management Regulatory Agency (PMRA)

In September 2007, the Canadian PMRA conducted a preliminary risk assessment of thiophanate-methyl. An acute RfD for the general population was set at 0.13 mg/kg bw. This was based on a NOAEL of 40 mg/kg bw/day for tremors that occurred within 2-4 h of dosing at 200 mg/kg/day in a 1-year study in dogs. A safety factor of 300 was applied, the additional

3 being for the lack of an acute neurotoxicity study in rodents. For females 13-50 years of age, an acute RfD of 0.067 mg/kg bw/d was set, based on a NOAEL of 20 mg/kg bw/d for supernumerary ribs in foetuses of exposed dams in a rabbit developmental toxicity study at 40 mg/kg/day. This effect was considered relevant to a single-dose exposure. The acute RfD incorporates a 300-fold uncertainty factor (the additional 3-fold for the lack of neurotoxicity and developmental neurotoxicity studies). A chronic RfD (analogous to an ADI) was set at 0.008 mg/kg bw/d, based on a NOAEL of 8 mg/kg bw/d for liver and thyroid toxicity in a 1-year dog study and using a 1000-fold uncertainty factor (increased 10-fold for lack of developmental neurotoxicity and endocrine disrupting compound studies).

European Commission

In February 2005, the European Commission conducted a review of the toxicology of thiophanate-methyl. An acute RfD of 0.2 mg/kg bw/d was set, based on a NOAEL of 20 mg/kg bw/d for supernumerary ribs in foetuses of exposed dams in a rabbit developmental toxicity study and using a 100-fold uncertainty factor (analogous to a safety factor). A chronic RfD (analogous to an ADI) was set at 0.08 mg/kg bw/d, based on a NOAEL of 8 mg/kg bw/d for liver and thyroid toxicity in a 1-year dog study and using a 100-fold uncertainty factor.

International Agency for Research on Cancer (*IARC)*

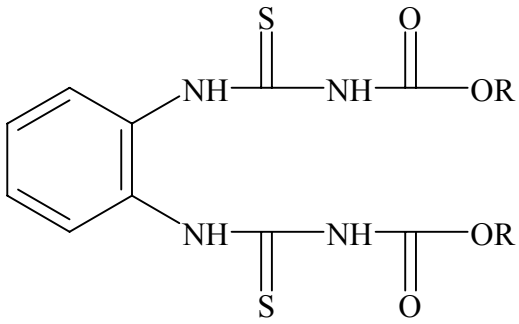
Thiophanate-methyl has not been evaluated by IARC.

International Programme on Chemical Safety (IPCS)

Thiophanate-methyl toxicology was evaluated by the IPCS of the WHO in 1973, 1975, 1977 and 1998. The ADI of 0.08 mg/kg bw/d allocated in 1973 was confirmed by the 1975 and 1977 Joint Meetings. In 1998, an ADI of 0.02 mg/kg bw/d was established on the basis of the NOAEL of 2 mg/kg bw/d for developmental toxicity in rabbits and a safety factor of 100. An AfRD was not set. The IPCS also concluded that thiophanate-methyl was adequately tested for genotoxicity and that thiophanate-methyl was not genotoxic.

1.3 Chemistry –Active Constituent

Common name:	Thiophanate-methyl (ISO Approved)
Chemical name:	Dimethyl 4,4’-(o-phenylene) bis (3-thioallophanate) (IUPAC)
CAS Registry Number:	23564-05-8
Empirical Formula:	$C_{12}H_{14}N_4O_4S_2$
Molecular Weight:	342.4
Chemical structure:	



Thiophanate-methyl R = CH_3

Chemical class: Benzimidazole

Structural analogues: Benomyl, Carbendazim

Chemical and physical properties

Colour:	White crystalline solid
Odour:	Odourless
Physical state:	Crystalline solid
Melting point:	Not determined, decomposes at 165ºC
Density (20^0C):	1.45m^3
Partition coefficient: (log P_{ow})	1.45
Vapour pressure:	<9.4 X 10^{-6} Pa at 9.5^{o}C
	<8.8 X 10^{-6} Pa at 19.6^{o}C
	<9.5 X 10^{-6} Pa at 29.8^{o}C
Solubility in water:	4 mg/L at pH 7 and 25^0C
Solubility in organic solvents:	
Hexane	0.47 mg/L
Xylene	110 mg/L
n-octanol	180 mg/L
Dichloromethane	730 mg/L
Acetone	29 g/L
Ethyl acetate	8.4 g/L
Methanol	7.8 g/L

Technical active

At the commencement of the review there was one approved holder for thiophanate-methyl.

Impurities of Toxicological Concern

The active thiophanate-methyl contains no impurities of toxicological concern, with the APVMA's minimum compositional standard specifying a minimum thiophanate-methyl content of 95%.

1.4 Products

At the time of this review, there were three registered thiophanate-methyl products in Australia: Banrot 400WP Broad Spectrum Fungicide for Ornamentals (containing 150 g/kg etridiazole and 250 g/kg thiophanate-methyl), Banrot 80G Broad Spectrum Fungicide for Ornamentals (containing 30 g/kg etridiazole and 50 g/kg thiophanate-methyl) and Zyban WSP Broad Spectrum Fungicide for Ornamental Plants (containing 640 g/kg mancozeb and 156 g/kg thiophanate-methyl). The three products are registered for the control of soil-borne diseases of ornamental plants and are applied either directly to the soil (evenly mixed with the soil) or as a spray. The labels do not indicate that these products are for home garden use.

2. METABOLISM AND TOXICOKINETICS

Tanoue T (1992) Thiophanate-methyl – Metabolism in rats. Environmental Toxicology Laboratory, Kanagawa, Japan. Study number: EC-338 Report date: 17 August 1992.

Test chemical:	[Phenyl-U-^{14}C]-Thiophanate-methyl (Lot No. C-109-2); Purity > 98.1%; Specific activity: 15 mCi/mmol] in corn oil and unlabelled Thiophanate-methyl (Lot No. TIF-1016; Purity 96.5.2%)
Test species:	Fischer F344 rats, 4-6 w.o from Charles River Japan Inc., Japan
GLP and QA:	Yes
Guidelines:	EPA 85-1

Material and methods : Rats (5/sex/dose) were given [Phenyl-U-^{14}C]-Thiophanate-methyl as a single oral dose of 14 (group A) or 170 mg/kg bw (group B), or an oral dose of 14 mg/kg bw (group C) following pre-treatment with unlabelled thiophanate-methyl at 14 mg/kg bw/d for 14 consecutive days (repeated doses). Blood samples were collected at 1, 2, 3, 4, 5, 6, 7, 9, 12, 24 and 48 hours after dosing. The faeces and urine were collected at 6 hr (urine only), 1, 2, 3 and 4 days after dosing. Expired air was not collected in this study since a preliminary study had shown that ^{14}C in expired air amounted to 0.01% or less of the dose. Rats were sacrificed 4 days after the radio labelled dose. The urine, cage-wash, faeces and tissue samples were radio assayed by liquid scintillation counting. In addition, metabolites from the urine and faeces were purified by HPLC and preparative TLC, and identified by NMR.

Absorption and excretion: Following administration of phenyl-U- ^{14}C- Thiophanate-methyl, the T_{max} in blood were 1-3h (low dose Groups A, C), 4-7h (high dose group B). C_{max} were 1.7-4.2 µg equivalent/g (µg/g) (groups A, C), 14-22 µg/g (Group D), and 17-27 µg/g, then decreased rapidly by 48h after dosing to <0.1 µg/g (groups A, C), <1 µg/g (group B). Concentrations of radioactivity in blood declined with terminal half-lives of 1.6-2.8h for groups A and C and 2.4-7.8h for group B.

Following a single low or high dose, or repeated doses, the majority (91-94%) of the dose was excreted within 24 hours and the excretion was complete (97-99%) within 48 hours (Table 2). For the low single dose, the predominant route of elimination was urine, but with approximately 25% in faeces while the reverse was observed for the high dose group. For the repeated low dose, ^{14}C excretion was similar for both routes. In all treated groups, urinary and faecal ^{14}C excretion was similar between males and females. Urinary excretion in the high dose group was somewhat less than after a single or repeated low dose after 48 hours, suggesting a slightly reduced gastrointestinal absorption.

Table 2: Excretion of [Phenyl-U-^{14}C]-Thiophanate-methyl (% of dose)

Dose (mg/kg bw)		**14 (single dose)**		**14 (repeated dose)**		**170 (single dose)**	
		male	***female***	***male***	***female***	***male***	***female***
0-24 hours	Faeces	25	24	44	43	63	67
	Urine	66	67	46	48	30	27
	Total	91	91	90	91	93	94
0-48 hours	Faeces	27	27	48	47	66	70
	Urine	70	71	50	51	32	29
	Total	97	98	98	98	98	99

Distribution: Radioactivity levels in the excised organs and tissues were generally very low at sacrifice on day 4 after administration (< 0.5% of the total administered dose) with the highest

level detected in the liver and thyroid. After 14-daily doses (14 mg/kg bw/d), the distribution of radioactivity showed a similar profile to that found after a single low dose (group A) indicating that thiophanate-methyl does not accumulate in tissues. No marked sex-related differences were observed in relation to tissue distribution in all groups. The radioactivity in blood and tissues in the single high dose group did not increase proportionally with the dose.

Metabolism: A large amount of the parent compound was detected in the faeces of the single high dose group (up to 56%) as well as in the repeated dose group (up to 24%); whereas only a small percentage of the parent compound was excreted in the faeces (around 1%) of the single low dose group (Table 3). The amount of the parent compound was negligible in the urine of any group (less than 0.5%). The main metabolite identified in the urine was 5-HBC-S for all three groups. The proposed metabolic pathway is represented in Figure 1.

Table 3: Main metabolites in rat urine and faeces (% of dose, 0-48 hours)

Metabolite	**Low dose**		**Repeat low dose**		**High dose**	
	male	***female***	***male***	***female***	***male***	***female***
Urine						
Thiophanate-methyl ™	0.2	0.5	0.2	0.4	0.2	0.4
4-OH-TM	2.4	2.1	1.5	1.2	1.1	0.7
Carbendazim	0.6	1.1	0.4	1.0	0.7	0.9
5-HBC-S	42	34	27	20	19	14
Faeces						
Thiophanate-methyl ™	1.1	1.1	24	21	52	56
4-OH-TM	10.4	8.4	6.5	5.7	4.1	3.7
Carbendazim	0.5	0.5	1.9	2.7	0.9	2.0
5-HBC-S	4.4	4.5	5.5	6.0	1.9	1.4

Nabetani M and Mori H (1993) Metabolism of 14C-Thiophanate-methyl in mice. Environmental Toxicology Laboratory, Kanagawa, Japan. Study number: EC-363 Report date: 21 May 1993.

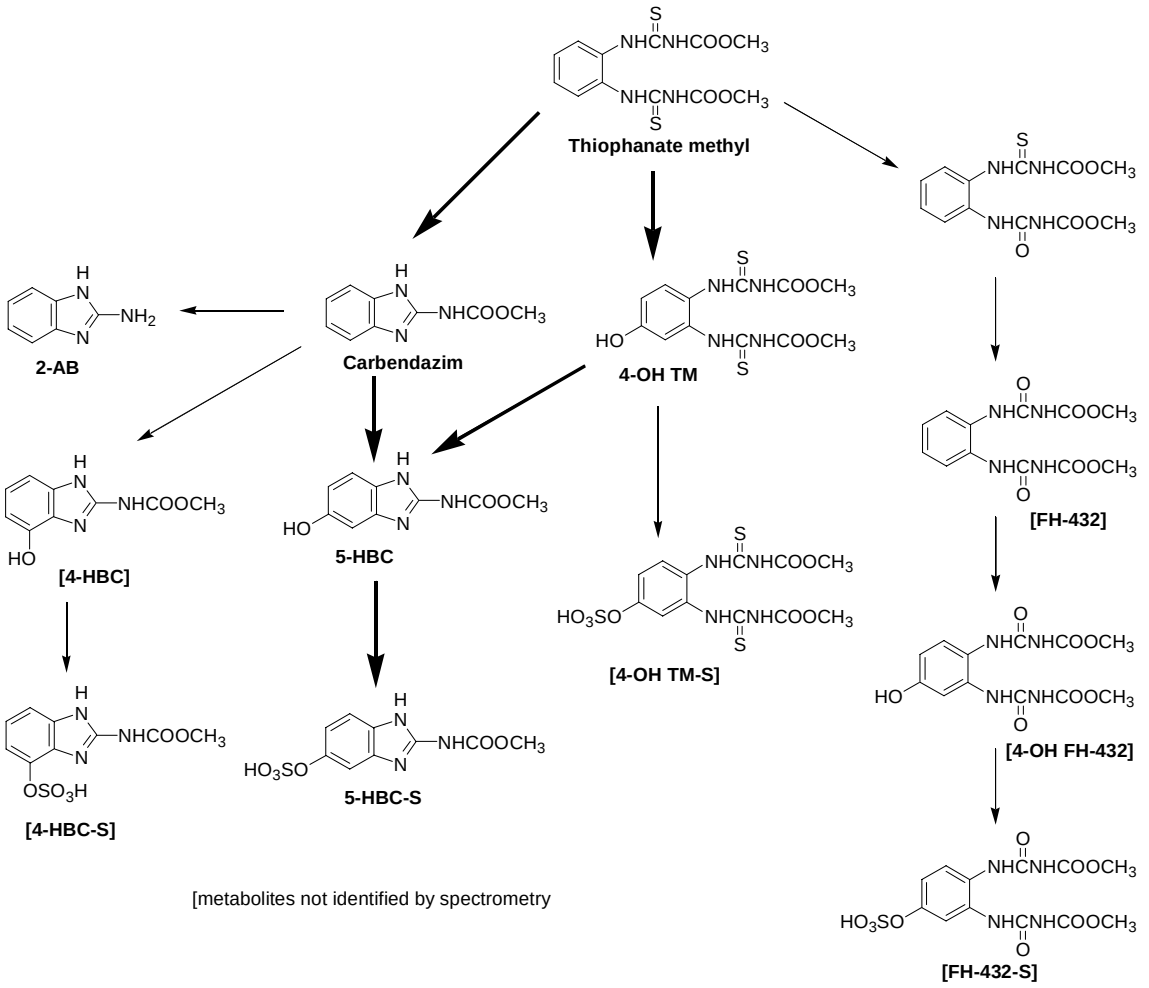
Test chemical:	[Phenyl-U-^{14}C]-Thiophanate-methyl (Lot No. C-109-2); Purity > 98%; Specific activity: 15 mCi/mmol] in corn oil and unlabelled Thiophanate-methyl (Lot No. TIF-1016; Purity 96.5.2%)
Test species:	CD-1 male mice, 5 w.o from Charles River Japan Inc., Japan
GLP and QA:	Yes
Guidelines:	EPA 85-1

Material and methods: CD-1 mice (5 males) were given [Phenyl-U-^{14}C]-Thiophanate-methyl as a single oral dose of 170 mg/kg bw. The faeces and urine were collected at 6 hr (urine only), 1, 2, 3 and 4 days after dosing. Expired air was not collected in this study since a preliminary study had shown that ^{14}C in expired air amounted to 0.01% or less of the dose. Rats were sacrificed 4 days after the radio labelled dose. The urine, cage-wash, faeces and tissue samples were radio assayed by liquid scintillation counting. In addition, metabolites from the urine and faeces were purified by HPLC and preparative TLC, and identified by NMR.

Results: Greater than 95% of the radioactivity was excreted in the urine (26%) and faeces (73%) with 24 hour after dosing. Less than 0.1% of the administered radioactivity remained in the body after 4 days. A large amount of the parent compound was detected in the faeces (up

to 48%) whereas the amount of the parent compound was negligible in the urine (0.1%). The main metabolite identified in the urine was 5-HBC-S (9%). It was concluded that the metabolism of thiophanate-methyl in mice is similar to that in rats. The amounts of carbendazim in urine and faeces are 0.5 and 6.3% respectively.

Figure 1: The proposed metabolic pathway in rats and mice (adapted from JMPR 1995: Carbendazim, Benomyl and Thiophanate-methyl: Pesticide residues in food)



Walters KA (1981) Percutaneous absorption of Thiophanate-methyl in rats. Fine Chemicals Research Laboratory, Nippon Soda Co., Ltd. Study No: RD-936 (unpublished.) Report date: 16 August 1993.

Test Compound: [^{14}C] Thiophanate-methyl (Purity ≥ 98%)
Test Species: CD male rats, 6 w.o, 199-220 g (source not stated)
GLP and QA: Yes
Guidelines: Not stated

Materials and methods: Anaesthetised male rats (4/dose) were given a dermal application of 0.3 mg/animal ^{14}C-Thiophanate-methyl (8 μg/cm^2 of Thiophanate-methyl) or 32 mg/animal (877 μg/cm^2 of thiophanate) to the clipped skin of the back (10 cm^2) under a non-occlusive protector. Rats were exposed for 0.5, 1.5, 10 or 24 hours. They were then sacrificed after washing the application site with soapy water (4 rats/time point). Urine (in the cage and from the bladder), faeces (in the cage and from the colon) were collected, while carcass and the application site skin were sampled. The radioactivity in the excreta and tissues including application site skin, as well as application site wash, protective appliance rinse and cage rinse was quantified by liquid scintillation.

Results: The total ^{14}C recovery was more than 99% from both doses (Table 4). The largest proportion of the dose (47-95%) was recovered from the application site wash. The *in vivo* dermal absorption increased with exposure time in both doses. Following a 24-hour exposure period, the *in vivo* dermal absorption of ^{14}C-Thiophanate-methyl from the low dose was around 53%, while absorption from the high dose was 23%.

Table 4: Radioactivity distribution (percentage of dose) in male rats (n=4)

Time after dosing (h)	*0.5*		*1.5*		*10*		*24*	
Dose (μg/cm^2)	*8*	*877*	*8*	*877*	*8*	*877*	*8*	*877*
Urine	0	0.0	0.3	0.1	11.3	2.64	25.18	9.8
Faeces	0.8	0.0	0.0	0.1	1.75	0.3	11	8.4
Carcass	4.5	2.5	4.1	1.8	15.4	5.8	16.2	5.2
Blood, liver, kidney	0.09	0.06	0.2	0.02	0.7	0.1	0.6	0.1
Washing/skin	95.4	97.3	95.3	97.8	70.8	91.3	47.1	76.3
Total recovery	100	99.8	99.9	99.8	99.9	100	99.5	99.8
***Total absorption**	4.6	2.7	4.6	2.2	29.2	8.7	53	23.6

*Total absorption was the sum of radioactivity in the urine, faeces, and carcass.

Walters KA (1993) In vitro skin penetration of Thiophanate-methyl. Fine Chemicals Research Laboratory, Nippon Soda Co., Ltd. Study No: RD-936 (unpublished.) Report date: 16 August 1993.

Test Compound: [^{14}C] Thiophanate-methyl (Purity: 98.3%)
Test Species: Post-mortem dermatomed human skin membrane and whole skin from male Wistar rats
GLP and QA: Yes
Guidelines: OECD 428

Materials and methods: Dermal permeability was measured over a 24-hour exposure period after a single application of 100 μL/cm^2 of 508 g/L ^{14}C-Thiophanate-methyl concentrate formulation (51 mg/cm^2) or (0.5 mg/cm^2) or (0.13 mg/cm^2) to excised human (n=3) and rat (n=6) skin membranes using static glass diffusion cells. The skin membranes were left

unoccluded for the duration of the study. Samples in receptor fluid (50% ethanol in water) were taken at 1, 2, 3, 4, 6, 8 and 24 hours and radioactivity was determined by liquid scintillation counting. Percutaneous absorption rates were calculated.

Results:

Rats
For all three preparations, the maximum absorption rates occurred between 0-8 hours. After a 24-hour exposure, total absorption was 0.51, 1.87 and 3.1% of the administered dose for the three preparations, respectively (Table 5).

Human
Similar to rat skin, the maximum absorption rates in human skin occurred at 8 hours and a 3 and 5-fold difference in absorption rates were seen for the three preparations. Consequently, total absorption after 24-hour exposure was significantly lower amounting to 0.16, 0.65 and 2.29% of the administered dose for the three preparations, respectively (Table 6).

Table 5: Absorption parameters in rat skin

	Mean absorption rates		*Mean percentage of dose absorbed*	
	Time (h)	*μg/cm²/h*	*Time (h)*	*Percentage*
Concentrate formulation (51 mg/cm²)	0-8 0-24	13.5 10.8	8 24	0.21 0.51
1:100 dilution (0.5 mg/cm²)	0-8 0-24	0.8 0.08	6 24	1.27 1.87
1:400 dilution (0.13 mg/cm²)	0-8 0-24	0.27 0.12	8 24	1.8 3.1

Table 6: Absorption parameters in human skin

	Mean absorption rates		*Mean percentage of dose absorbed*	
	Time (h)	*μg/cm²/h*	*Time (h)*	*Percentage*
Concentrate formulation (51 mg/cm²)	0-8 0-24	4.4 3.4	8 24	0.07 0.16
1:100 dilution (0.5 mg/cm²)	0-8 0-24	0.16 0.14	8 24	0.26 0.65
1:400 dilution (0.13 mg/cm²)	0-8 0-24	0.07 0.09	8 24	0.43 2.29

3. ACUTE TOXICITY STUDIES

3.1 Active constituent

3.1.1 Acute oral and dermal toxicity

The results of acute oral and dermal toxicity studies conducted on technical thiophanate-methyl (96.55% purity) is summarised in the Table below.

Species	Guidelines	Vehicle	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral acute toxicity					
Crj:CD (SD) Rat 5/sex	OECD No. 401	Distilled water	5000 Oral gavage	>5000 No toxic signs or deaths	Nishibe T (1990a)
Dermal acute toxicity					
Kbs:JM rabbits 5/sex/group	OECD No. 402	Distilled water	0 (control) and 2000 single occlusive application for 24h	>2000 Erythema in 7 animals for 2 days. No other toxic signs No deaths	Nishibe T (1990b)

3.1.2 Skin irritation

Nishibe T. (1986) Thiophanate-methyl: Primary dermal irritation study in rabbits. Laboratory: not specified. Report No: Doc. 565-002, RD-8692. Study duration: 21 July – 25 July 1986. Report date: not specified. Guidelines: OECD 404. GLP: Yes.

Materials and Method: Six New Zealand White rabbits (male, 3 months old; 3.2 kg±0.20 kg) were acclimatised for 1 week prior to a single application of thiophanate-methyl (96.2% purity) at 0.5 g. The test substance was moistened with water then applied to shaved intact dorsal skin area of the trunk of the animal by means of a lint patch (3 cm^3) under semi-occlusive conditions. After an exposure period of 4 hours, the patch was removed and the skin was wiped to remove any residual test material. Skin irritation (erythema and oedema) was evaluated at 0, 0.5, 1, 24, 48 and 72 hours after patch removal. The degree of irritation was scored according to the Draize Scale. As no skin reaction was observed at any observation points, the study was terminated 72 h after removal.

Results: Since no skin reaction was observed in any animal during the observation period, it was concluded that thiophanate-methyl is not a skin irritant in rabbits.

3.1.3 Eye irritation

Nishibe T. (1986b) Thiophanate-methyl: Primary eye irritation study in rabbits. Laboratory: not specified. Report No: Doc. 566-002, RD-8691. Study duration: 21 July - 24 July 1986. Report date: not specified. Guidelines: OECD 405. GLP: Yes.

Materials and Method: Nine New Zealand White rabbits (male, 3 months old; 2.91 kg±0.19 kg) were acclimatised for 1 week prior to a single application of thiophanate-methyl (96.2% purity) at 0.1 g. The test substance was moistened with water, and then instilled into the sac of one eye per rabbit. The untreated eye served as control. In 3 out of 9 rabbits, the treated eyes were rinsed 2 minutes after treatment for 30 seconds. Reading for eye lesions was made at 1, 24, 48 and 72h after dosing. As there were no findings 48h after treatment, the experiment was terminated after 72h.

Results: Initial redness and/or chemosis were observed in the majority of animals but the signs persisted in only 1 rabbit at 24h then disappeared at 48h. There were no difference between rabbits whose eyes were rinsed and those remained un-rinsed after exposure. No ocular reaction was observed in any rabbit. It was concluded that thiophanate-methyl is not an eye irritant in rabbits.

3.1.4 Acute Inhalation toxicity

Nishibe T (1987) Thiophanate-methyl: Acute inhalation study in rats (4-hour exposure). Laboratory: not specified. Report No: Doc. 523-001, RD-8711. Study duration: 30 September 1986 – 24 February 1987. Report date: not specified. Guidelines: OECD 403. GLP: Yes.

Materials and method: Rats (Crj:CD SD, 5 M & 6F/group; 5 groups, 5 weeks old, 146-183g) were acclimatised to standard laboratory conditions for 1 week prior to the start of the study. Food and water were available *ad libitum* except during the exposure period. Thiophanate-methyl (95.3% purity) was administered by whole body exposure for 4 hours at chambers to actual dust concentration of 0, 1.0, 1.5, 1.6 or 1.9 mg/l air, and 5 females were additionally exposed to 0.5 mg/l. Whether or not 1.9 mg/l was the highest attainable dust concentration was not stated. The mean median particle diameters were 3.7-4.5 μm and most particles were smaller than 10 μm in diameter. Mortality and clinical signs were recorded at 1 and 3 hours after exposure, upon removal from the chamber and then once daily for 14 days. Bodyweight was measured prior the study and on days 1, 2, 3, 7 and 14. Gross necropsy was performed at the termination of the experiment at day 14 on all rats that died during the observation period or the survived until termination of the experiment.

Results: All male and 60% of female rats died at concentration of 1.9 mg/l but no death in other dose groups (except 1 animal at 1 mg/l dose group) indicating the mortality was dose-related. Toxic signs included decreased motor activity, low sensitivity, ataxia, ptosis, urinary incontinence, tremor, convulsion, hypotonia and ventral position. The bodyweight decrease and growth depression were observed for 1-3 days after the exposure in many rats but their bodyweight increased thereafter. At necropsy one rat died at 1 mg/l dose group showed dark reddish lungs.

The LC_{50} (4-h) was 1700 mg/m^3 for males and 1900 mg/m^3 for females, therefore it was of moderate acute inhalation toxicity in rats.

3.1.5 Skin sensitisation

Nishibe T (1989) Thiophanate-methyl: delayed contact hypersensitivity study in Guinea pigs. Laboratory: not specified. Report No: Doc. 567-001, RD-8924. Study duration: 26 May – 18 July 1986. Report date: not specified. Guidelines: OECD 406. GLP: Yes.
Materials and methods: The sensitising potential of thiophanate-methyl (96.2% purity) was investigated using Guinea pig maximization test according to Magnusson and Kligmann. The dose selection was appropriate. In the test group, Hartley guinea pigs (20 females, 6 months old, 387.1±20.9g) was given intradermal induction by injection of 3.5% TM in corn oil on Day 1, then dermal induction for 48h with 42% TM in white Vaseline on Day 8, followed by dermal challenge for 24h with 42% TM in white Vaseline on Day 21. 2,4,Dinitro-chloro-benzene (DNCD) was used as positive control. 0.1% in corn oil was used for intradermal induction, 1% in white Vaseline was used for both dermal induction and challenge. The number of animals used for positive control was not specified.

Results: The positive control animals showed skin reactions as expected. In the test group, all animals showed skin reactions 24h after patch removal and 19/20 animals also 48h and 72h after patch removal. It was concluded that TM is a skin sensitizer under the test conditions in Guinea pigs. Note that the severity of the skin reactions was not reported by the applicant.

Nishibe T., Mochizuki N., (1993). Product Topsin M 500 SC: Skin sensitisation study in Guinea pigs. Laboratory: not specified. Report No: Doc. 567-002, RD-9347. Study duration: 13 July – 19 August 1993. Report date: not specified. Guidelines: OECD 406. GLP: Yes.

Materials and methods: In the main study, 30 Guinea pigs were divided into 3 groups (10/group, sex not specified, 5-6 weeks old, 336-423 g) for test substance (30% TM in saline), positive control (0.5% DNCB in ethanol) and negative control. Negative control animals were untreated during the induction phase. On Day 0, the left flank of the animals were clipped free of hair and 0.2 mL of the test substance or DNCB were applied to 4cm2 skin for 6h under occlusive condition. The second and third inductions were conducted to the same test site in similar manner on Days 7 and 14. The animals were then challenged on Day 28. The right flank of the animals were clipped free of hair and 0.2 mL of the test substance or DNCB were applied to 4cm2 skin for 6h under occlusive condition.

The challenge sites were evaluated 24h, 48h and 72h after the application of the patches.

Results: Positive control animals showed slight to moderate erythema at 24h and 48h after removal of the patch. No skin reactions were observed in the control and treated groups. Therefore, it was concluded that Thiophanate-methyl was not a skin sensitizer under the conditions of Buhler test.

3.2 Products/formulations

The results of acute toxicity studies conducted on thiophanate-methyl products similar to the three Australian products are evaluated below (see Appendix IV for the formulation details of these products). Banrot® 80G Broad Spectrum Fungicide for Ornamentals is a ready to use granule formulation that will be used in the control of damping–off and other root diseases. Its formulation is similar to that of Banrot® 400WP Broad Spectrum Fungicide for Ornamentals

except that the concentrations of the active constituents are reduced by about 80%. Acute oral, dermal and inhalation toxicity, skin and eye irritation and skin sensitisation studies were conducted on Banrot® 40% WP Fungicide, which is identical to Banrot® 400WP Broad Spectrum Fungicide for Ornamental Plants. Eye irritation was also assessed on a 0.09% aqueous suspension of Banrot® 40% WP Fungicide. Zyban WSB Broad Spectrum Fungicide for Ornamental Plants may be used for the control of a range of foliar diseases of ornamental plants. No food crops are listed on the labels. The results of these studies are summarised in Table 7.

Table 7: Summary of acute toxicity of thiophanate-methyl products

Banrot® 400WP & Banrot® 80WP Broad Spectrum Fungicide for Ornamental Plants (250 or 50 g/kg Thiophanate-methyl)				
Test Chemical	**Species**	**Study**	**Result**	**Reference**
Banrot® 40% WP Fungicide	Rat (♂ + ♀)	Acute Oral	LD_{50} >5000 mg/kg bw	Schindler-Horvat (1993a)
Banrot® 40% WP Fungicide	Rabbit (♂ + ♀)	Acute Dermal	LD_{50} >2000 mg/kg bw	Schindler-Horvat, (1993b)
Banrot® 40% WP Fungicide	Rat (♂ + ♀)	Inhalation	LC_{50} >4480 mg/m^3 (zero deaths at this dose)	Labbé (1993)
Banrot® 40% WP Fungicide	Rabbit (♂ + ♀)	Dermal Irritation	Moderate	Schindler-Horvat (1993c)
Banrot® 40% WP Fungicide	Rabbit (♂ + ♀)	Eye Irritation	Moderate	Schindler-Horvat (1993d)
0.09% Aqueous Suspension of Banrot® 40% WP Fungicide	Rabbit (♂ + ♀)	Eye Irritation	Non–irritant	Schindler-Horvat (1993f)
Banrot® 40% WP Fungicide	Guinea pig (♂ + ♀)	Skin Sensitisation (Buehler Closed Patch Method)	Non–sensitiser	Schindler-Horvat (1993g)
ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants (150 g/kg Thiophanate-methyl)				
ZYBAN WSB	Rat (SD)	Acute Oral	LD_{50} >5000 mg/kg bw	Gabriel (1995a)
ZYBAN WSB	Rabbit (NZ White)	Acute Dermal	LD_{50} >2000 mg/kg bw	Gabriel (1995b)
ZYBAN WSB	Rat (SD)	Inhalation	LC_{50} >2427 mg/m^3	Hershman (1995)
ZYBAN WSB	Rabbit (NZ White)	Dermal Irritation	Not a skin irritant	Moore (1995a)
ZYBAN WSB	Rabbit (NZ White)	Eye Irritation	Moderate	Moore (1995b)
ZYBAN WSB	Guinea pig	Skin Sensitisation (Buehler Closed Patch Method)	Non–sensitiser	Moore (1995c)

Schindler-Horvat, J.E. (1993a) Banrot® 40% WP fungicide: Acute oral (limit) toxicity study in male and female rats. Grace Sierra Crop Protection Company, PO BOX 4003, 1001 Yosemite Drive, Milpitas, CA 95035-2003, USA. Sri International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA. 16 December 1993. Sri study no. lsc 3798-mo43-92.

Test Chemical	Banrot® 40% WP Fungicide, lot no. 4322R163, 15.2% etridiazol,26.9%thiophanate–methyl
Dose and Route	5000 mg/kg bw orally by gavage
Suspension	25% (w/v) in deionised water
Dose Volume	20 mL/kg bw
Test Species	5 ♂ (8 weeks old, 227–245 g) and 5 ♀ (11 weeks old, 203–230 g) Sprague–Dawley rats (From Simonsen Laboratories, 1180 C Day Road, Gilroy, CA 95020, USA)
Duration of Study	28 October 1992–11 November 1992
QA/GLP	Yes
Guidelines	OECD (401) US EPA (40 CFR Part 158, 81–1)

Materials and methods: Five rats/sex were fasted overnight and given a single oral dose of Banrot® 40% WP Fungicide (5000 mg/kg bw) by gavage. Rats were housed 5 per cage in stainless steel cages, the housing environment was controlled and monitored and food and water were available *ad libitum*, except for the period of fasting. Animals were observed for mortality and clinical signs of toxicity at approximately 0.3, 1, 4 and 6.5 hours after dosing and at least once daily thereafter for 14 days. Bodyweight was recorded on day 0 (prior to dosing) and on days 7 and day 14. All animals were sacrificed on day 14 and visual examinations were made of the external structures, including body orifices. Cranial, thoracic and abdominal organs were examined *in situ*.

Results: One male and 1 female were found dead on day 2. Ataxia, convulsions, decreased spontaneous motor activity, exudate on muzzle and forelimbs, humped back, rough coat, tremors and weakness were observed in 1–10 rats up until day 8. Dehydration and emaciation were observed in 1–4 females on days 7–8. One female lost bodyweight (1%), but the remaining animals gained weight (25–30% in males and 1–5% in females) over the 14 day study period. Macroscopic pathological findings, such as a white solid in the lumen of the bladder, dark thymus, enlarged uterine horns and dark red lungs were each observed in 1 animal. The acute oral LD_{50} for Banrot® 40% WP Fungicide is >5000 mg/kg bw in male and female rats.

Schindler-Horvat, J. E. (1993b) Banrot® 40% WP Fungicide: Acute Dermal (Limit) Toxicity Study in Male and Female Rabbits. Grace Sierra Crop Protection Company, PO Box 4003, 1001 Yosemite Drive, Milpitas, CA 95035-2003, USA. SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA. 16 December 1993. SRI Study No. LSC 3798-MO44-92.

Test Chemical	Banrot® 40% WP Fungicide, lot no. 4322R163, 15.2% etridiazol, 26.9% thiophanate–methyl
Dose	2000 mg/kg bw
Test Species	5 ♂ (2.73–3.07 kg) and 5 ♀ (2.49–3.26 kg) adult New Zealand White rabbits (From Western Oregon Rabbit Company, PO Box 653, Philomath, OR 97370, USA)
Duration of Study	28 October 1992–11 November 1992
QA/GLP	Yes
Guidelines	OECD (402) US EPA (40 CFR Part 158, 81–2)

Materials and methods: One day before treatment, the fur was clipped free from the dorsal area of the trunk of each rabbit (5 rabbits/sex) with electric clippers. Banrot® 40% WP Fungicide (2000 mg/kg bw) was applied directly to a 10 cm × 15 cm area of clipped intact skin and was moistened with saline (2 mL for every gram of test material). The test material was held in contact with the skin by a porous gauze dressing and non–irritating tape. The entire trunk of each animal was wrapped in elastic tape. Each animal was fitted with an Elizabethan collar to prevent access to the patch. After 25 hours the collar and bandages were removed, the test substance was wiped with paper towels moistened with warm tap water and the site was shaved. Rabbits were individually housed in stainless steel cages, the housing environment was controlled and monitored and food and water were available *ad libitum*. Animals were observed for mortality, clinical signs of toxicity or reaction to treatment at approximately 0.75 and 2.1 hours after dosing and at least once daily thereafter for 14 days. Bodyweight was recorded on day 0 (prior to dosing) and on days 7 and day 14. All animals were sacrificed at the end of the study and visual examinations of the application site and external structures were made, including body orifices. Cranial, thoracic and abdominal organs were examined *in situ*.

Results: There were no mortalities during the study period. Redness was observed in 4–10 animals from days 1–14 and oedema was observed in 7–10 animals on days 1–12. There were 5 and 1 animals with oedema on days 13 and 14, respectively. Exfoliation, including flaking skin, superficial open lesions, scabs and peeling skin were observed from day 3 in 4–10 animals. Broken skin (2–5 rabbits on days 5–12) and thickened skin (4–9 rabbits on days 4–12) were also observed. All rabbits gained weight (3–15% in males and 6–29% in females) during the study. The acute dermal LD_{50} for Banrot® 40% WP Fungicide is >2000 mg/kg bw in male and female rabbits.

Labbé, R. (1993c). An Evaluation of the Acute Toxicity of an Inhaled Aerosol of Banrot® 40% Wettable Powder Broad Spectrum Fungicide in the Albino Rat (Safety Test). Grace Sierra Crop Protection Company, PO Box 4003, 1001 Yosemite Drive, Milpitas, CA 95035-2003, USA. Bio-Research Laboratories Ltd, 87 Senneville Road, Senneville, Quebec H9X 3R3, Canada. 2 June 1993. Bio-Research Project No. 90632.

Test Chemical	Banrot® 40% WP Fungicide, lot no. 4322R1S3, 15.1% etridiazol, 26.5% thiophanate–methyl
Dose	4480 mg/m^3
Test Species	10 ♂ (254–282 g) and 10 ♀ (182–194 g) Sprague–Dawley CD [Crl:CD®(SD)BR] strain rats (65 days old) (From Charles River Canada, St Constant, Quebec, Canada)
Duration of Study	14 October 1992–28 October 1992
QA/GLP	Yes
Guidelines	OECD (403) US EPA (40 CFR Part 160, 81–3)

Materials and methods: Five rats/sex received a nose only inhalation exposure to Banrot® 40% WP Fungicide (4480 mg/m^3) or air (control). The exposure chamber (approximately 43 L) was operated at an airflow rate of 80–90 L/min during the treatment. The t_{95} (equilibrium time for aerosol concentration to reach 95% of the target concentration) was 1.6 minutes. Rats were housed individually in stainless steel cages, the housing environment was controlled and monitored and food and water were available *ad libitum*, except during inhalation treatment and prior to necropsy when food was withheld. Animals were observed twice daily for mortality and on the day of treatment. All animals were examined for clinical signs of toxicity on the day of treatment (pre– and post–exposure), twice daily for the next 13 days and prior to necropsy. Bodyweight was recorded on day 1 (day of treatment) and on days 2, 3, 4, 7 and 14. At the end of the 14 day observation period, all animals were fasted overnight, sacrificed and given a macroscopic pathological examination. The necropsy consisted of a detailed external and internal examination, with particular attention to the respiratory tract, skin and eyes. The lungs of each animal were dissected free of fat and weighed. The bronchi, kidneys, larynx, liver, lungs (all lobes), nasal cavities and sinuses, pharynx, trachea and abnormal tissues were preserved for microscopic examination.

Results: The mean mass aerodynamic diameter was 5.1 μm and the 25% particle size by mass was estimated to be 3 μm. There were no mortalities during the study. Twitching (all rats) and slight salivation (5 males and 2 females) were observed on day 1 after treatment. Wheezing was observed on days 2–4 in 1–5 males and 1–2 females. There were no clinical signs in any control rat. Bodyweight loss was recorded in 10/10 treated animals and 3/10 control animals on the day after treatment. However, all control (34.6% in males and 21% in females) and treated (32% in males and 17% in females) rats gained bodyweight over the entire 14 day study period. The mean absolute lung weight (1.321–1.372 g in males and 1.071–1.075 g in females) and the mean lung: bodyweight ratio (0.421–0.446 in males and 0.540–0.555 in females) was comparable between control and treated rats. Dark (2/5) and depressed (1/5) areas were observed in the lungs of treated males. There were no treatment–related microscopic pathological findings. The acute inhalation LC_{50} for Banrot® 40% WP Fungicide is >4480 mg/m^3 in male and female rats.

Schindler-Horvat, J. E. (1993d). Banrot® 40% WP Fungicide: Primary Dermal Irritation Study in Rabbits. Grace Sierra Crop Protection Company, PO Box 4003, 1001 Yosemite Drive, Milpitas, CA 95035-2003, USA. SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA. 16 December 1993. SRI Study No. LSC 3798-MO45-92.

Test Chemical	Banrot® 40% WP Fungicide, lot no. 4322R163, 15.2% etridiazol, 26.9% thiophanate–methyl
Dose	500 mg
Test Species	3 ♂ (2.94–3.14 kg) and 3 ♀ (2.50–2.66 kg) adult New Zealand White rabbits (From Western Oregon Rabbit Company, PO Box 653, Philomath, OR 97370, USA)
Duration of Study	3 November 1992–18 November 1992
QA/GLP	Yes
Guidelines	OECD (404) US EPA (40 CFR Part 158, 81–5)

Materials and methods: One day before treatment, the fur was clipped free from the dorsal area of the trunk of each rabbit (3 rabbits/sex) with electric clippers. Banrot® 40% WP Fungicide (powdered solid, 500 mg) was moistened with 1 mL of saline and applied to a ~ 5 cm × 5 cm area of clipped unabraded skin. The test material was applied under a gauze patch and held in place with non–irritating tape. The patch was loosely held in contact with the skin by an occlusive dressing for 4 hours. Each animal was fitted with an Elizabethan collar to prevent access to the patch. After 4 hours the collar and bandages were removed, the test substance was wiped with paper towels moistened with warm tap water. Dermal irritation (Draize score) was assessed at approximately 30–60 minutes and 24, 48 and 72 hours and 7 and 14 days after patch removal. Clinical signs of toxicity were also noted and animals were observed daily for mortality. Rabbits were individually housed in stainless steel cages, the housing environment was controlled and monitored and food and water were available *ad libitum*.

Results: There were no mortalities or clinical signs during the study period. At 1 hour after patch removal, all animals had erythema (score = 1 in 4 rabbits and score = 2 in 2 rabbits). Erythema occurred in 4 rabbits at 24 (score = 1 in 2 rabbits and score = 2 in 2 rabbits), 48 (score = 1 in 2 rabbits and score = 2 in 2 rabbits) and 72 (score = 1 in 3 rabbits and score = 2 in 1 rabbit) hours and 7 days (score = 1 in 3 rabbits and score = 2 in 1 rabbit). Oedema (score =1) was observed at 1 (2 rabbits) and 24 (1 rabbit) hours and 7 days (1 rabbit) after patch removal. Since erythema (up to score = 2) and oedema (score = 1) were observed 7 days after treatment, Banrot® 40% WP Fungicide is a moderate skin irritant in male and female rabbits.

Schindler-Horvat, J. E. (1993e). Banrot® 40% WP Fungicide: Primary Eye Irritation Study in Rabbits. Grace Sierra Crop Protection Company, PO Box 4003, 1001 Yosemite Drive, Milpitas, CA 95035-2003, USA. SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA. 16 December 1993. SRI Study No. LSC 3798-MO46-92.

Test Chemical	Banrot® 40% WP Fungicide, lot no. 4322R163, 15.2% etridiazol, 26.9% thiophanate–methyl
Dose	100 mg
Test Species	3 ♂ (2.88–3.34 kg) and 6 ♀ (2.84–3.30 kg) adult New Zealand White rabbits (From Western Oregon Rabbit Company, PO Box 653, Philomath, OR 97370, USA)
Duration of Study	10 November 1992–15 December 1992
QA/GLP	Yes
Guidelines	OECD (405) US EPA (40 CFR Part 158, 81–4)

Materials and methods: Banrot® 40% WP Fungicide (100mg) was placed in the conjunctival sac of the right eye of each rabbit (3/sex) as a powdered solid, after gently pulling the lower lid away from the eyeball. The eyelids were then held together for approximately 1 second. Three additional females were treated following the same procedure, except that treated eyes were gently flushed with room–temperature water for 30 seconds, 30 seconds after treatment. At 24 hours after treatment the "unwashed–eyes" of the treated rabbits (3/sex) were gently washed with saline. The treated eye of one "unwashed–eye" rabbit was also rinsed at 48 hours. Eyes were examined at 1, 24, 48 and 72 hours and 7 and 14 days after treatment. After the 72 hour observation, the eyes of all "washed–eye" rabbits and 3/6 "unwashed–eye" rabbits were examined with the aid of 0.25% fluorescein sodium ophthalmic solution. Rabbits were assessed for corneal, iridal and conjunctival effects using the Draize score and for clinical signs of toxicity. At the end of the 14 day observation period, all animals were sacrificed. Rabbits were individually housed in stainless steel cages, the housing environment was controlled and monitored and food and water were available *ad libitum*.

Results: There were no mortalities or clinical signs during the study. In the unwashed eyes, corneal opacity (score = 1 in 2 rabbits), and conjunctival redness (score = 2 in 3 rabbits and score = 3 in 3 rabbits), chemosis (score = 2 in 3 rabbits and score = 3 in 3 rabbits) and discharge (score = 3 in 6 rabbits) were observed after 1 hour. Corneal opacity (score = 1–2) was observed for 72 hours in 4 rabbits and for 7 days (score = 1) in 2 rabbits. Iridal (score = 1) effects were observed in 2 rabbits at 24 hours and 1 rabbit at 72 hours. Conjunctival redness (score = 1–2) lasted up to 72 hours in 5/6 rabbits and for 7 days in 2 rabbits. Chemosis (score = 1–3) persisted for 48 hours in all animals and was observed in 1 rabbit at 7 days (score = 1) and discharge had ceased in all but 1 animal at 72 hours (score = 1). In the 3 rabbits with washed eyes, corneal opacity (score = 1) was present in all animals at 1 hour and in 1 rabbit at 72 hours. There were no iridal effects, but conjunctival redness (score = 2–3), chemosis (score = 1–2) and discharge (score = 1–2) were observed at 1 hour. Redness persisted in all rabbits until day 72 (score = 1) and discharge (score = 1) occurred in 1 rabbit at 72 hours. Based on the irritation scores in the "unwashed–eyes" of rabbits, Banrot® 40% WP Fungicide is a moderate eye irritant in male and female rabbits.

Schindler-Horvat, J. E. (1993g).Banrot® 40% WP Fungicide-Use Dilution: Primary Eye Irritation Study in Rabbits. Grace Sierra Crop Protection Company, PO Box 4003, 1001 Yosemite Drive, Milpitas, CA 95035-2003, USA. SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA. 16 December 1993. SRI Study No. LSC 4179-MO58-92.

Test Chemical	0.09% aqueous suspension of Banrot® 40% WP Fungicide, lot no. 4322R163, 15.2% etridiazol, 26.9% thiophanate–methyl
Dose and Route	0.1 mL
Test Species	3 ♂ (2.33–2.78 kg) and 6 ♀ (2.92–3.03 kg) adult New Zealand White rabbits (From Western Oregon Rabbit Company, PO Box 653, Philomath, OR 97370, USA)
Duration of Study	1 December 1992–4 December 1992
QA/GLP	Yes
Guidelines	OECD (405) US EPA (40 CFR Part 158, 81–4)

Materials and methods: A 90mg/100 mL (deionised water) suspension of Banrot® 40% WP Fungicide (0.1 mL) was applied to the conjunctival sac of the right eye of each rabbit (3/sex) after gently pulling the lower lid away from the eyeball. The sponsor indicated that this dose was chosen because it is the most concentrated use dilution recommended on the Banrot® package label. The eyelids were then held together for approximately 1 second. The left eye served as a control. Eyes were examined at 1, 24, 48 and 72 hours and 7 and 14 days after treatment. Rabbits were assessed for corneal, iridal and conjunctival effects using the Draize score and for clinical signs of toxicity. At the end of the 72 hour observation period, all animals were sacrificed. Rabbits were individually housed in stainless steel cages, the housing environment was controlled and monitored and food and water were available *ad libitum*.

Results: There were no mortalities or clinical signs during the study. Conjunctival redness (score = 1) was observed in 1/6 rabbits at 24 and 48 hours and chemosis was observed in 1/6 rabbits at 24 hours. There were no other signs of ocular irritation. Under the conditions of the study the 0.09% aqueous suspension of Banrot® 40% WP Fungicide is not an eye irritant in male and female rabbits.

Schindler-Horvat, J. E. (1993f). Banrot® 40% WP Fungicide: Skin Sensitisation Study in Guinea Pigs. Grace Sierra Crop Protection Company, PO Box 4003, 1001 Yosemite Drive, Milpitas, CA 95035-2003, USA. SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA. 16 December 1993. SRI Study No. LSC 3798-MO47-92.

Test Chemical	Banrot® 40% WP Fungicide, lot no. 4322R163, 15.2% etridiazol, 26.9% thiophanate–methyl
Dose	25–100% w/v in distilled water
Positive Control	Dinitrochlorobenzene (DNCB)
Dose	0.2–0.4% w/v in 80% aqueous ethanol
Test Species	23 ♂ (305–365 g) and 23 ♀ (282–322 g) ♂ Hartley albino guinea pigs (6 weeks old) (From Charles River Laboratories, Portage, MI, USA)
Duration of Study	1 September 1992–9 October 1992
QA/GLP	Yes
Guidelines	OECD (406) US EPA (40 CFR Part 158, 81–6)

Materials and methods: In a dose–range study, guinea pigs (2/sex) received dermal applications of 25–75% Banrot® 40% WP Fungicide w/v in deionised water and 100%

Banrot® 40% WP Fungicide (400 mg moistened with deionised water). From this study, the concentration of the test material used for induction was 25% and for the challenge was 50%.

In the induction phase of the definitive study (Buehler Topical Closed Patch Technique), the left flank of guinea pigs (5/sex) was clipped free of hair the day before application of 0.4 mL of a 25% w/v suspension of Banrot® 40% WP Fungicide. Inductions were made at the same site once weekly for 3 weeks. The test material was applied to a 20 mm × 20 mm pad, which was held in place on the test site by tape and occluded with a rubber dam. The torso was wrapped in an elastic adhesive tape, but animals were not restrained. A negative (deionised water, 5/sex) and positive (0.4% DNCB in 80% aqueous ethanol, 3/sex) control, and the positive control vehicle (or 80% aqueous ethanol, 3/sex) were also assessed using the procedure described above. After 6 hours exposure, wraps were removed and remaining test substance was removed with tap water and paper towels. Twenty four hours later, the application sites were scored for erythema.

Fourteen days after administration of the 3rd induction dose, all guinea pigs were challenged topically with Banrot® 40% WP Fungicide (0.4 mL of a 25% w/v suspension) on a previously untreated site on the right flank. Challenge sites were occluded for 6 hours as described previously. Nineteen hours after removal of patches, challenge sites were depilated and at 24 and 48 hours after patch removal, skin reactions were assessed. One week after the challenge, test animals were re–challenged with Banrot® 40% WP Fungicide (0.4 mL of a 25% w/v suspension) on a previously unexposed area of the right flank. Naïve controls (5/sex) received the re–challenge on both flanks. Treatment followed the same procedure as for the challenge application.

Animals were observed for mortality and clinical signs of toxicity twice daily (once daily on weekends and public holidays). Bodyweights were recorded before the 1st induction and before the challenge application. During the study, guinea pigs were individually housed in polycarbonate cages, the housing environment was controlled and monitored and food and water were available *ad libitum*.

Results: There were no mortalities or abnormal clinical signs. One treated female (5%) and one ethanol control female (6%) experienced only slight bodyweight gains during the study period. Otherwise, mean group bodyweight gains were comparable between all groups (46–55% in males and 32–45% in females).

In treated animals, very faint (score = 0.5) erythema was observed 24 hours after the 1st (1/10 animals), 2nd (2/10 animals) and 3rd (4/10 animals) inductions, with faint (score = 1) erythema observed after the 2nd (4/10 animals) and 3rd (3/10 animals) inductions. DNCB–induced animals showed very faint (score = 0.5) to strong (score = 3) erythema following induction. There were no signs of irritation after any induction in the control animals.

After the challenge application to treated animals, very faint (score = 0.5) erythema was observed at 24 (2/10 animals) and 48 (6/10 animals) hours, with faint (score = 1) erythema observed at 24 (2/10 animals) and 48 (1/10 animals) hours. Moderate (score = 2) erythema was observed in 1/10 animals at 24 hours. Focal scabs were present (1/10 at 24 and 48 hours and 3/10 at 48 hours only), with scabbing and a focal whitish pink area observed in the animal with moderate erythema. In control animals, very faint (score = 0.5) erythema was observed in 1/10 animals at 24 hours and 5/10 at 48 hours, with faint (score = 1) erythema observed in 1/10 animals at 48 hours. Focal white pink areas and scabbing occurred in the control animal

with faint erythema and focal scabbing at the application site was observed in 2 other control animals at 48 hours. In DNCB–challenged guinea pigs, very faint (score = 0.5) to moderate (score = 2) erythema was observed in all animals at 24 hours, but erythema (score = 1 in 2 guinea pigs and score = 3 in 2 guinea pigs) was only observed in 4/6 animals (1/3 males) at 48 hours.

Following re–challenge to the treated animals, very faint (score = 0.5) to faint (score = 1) erythema was observed in 9/10 guinea pigs at 24 and/or 48 hours. In control animals, very faint (score = 0.5) erythema was observed in 3/10 animals at 48 hours and faint (score = 1) erythema was observed in 1/10 animals at 24 and 48 hours.

Mean Irritation Scores							
Group	**Induction**			**Challenge**		**Re–Challenge**	
	1st	**2nd**	**3rd**	**24 Hour**	**48 Hour**	**24 Hour**	**48 Hour**
Control							
♂	0	0	0	0.2	0.3		
♀	0	0	0	0.1	0.2		
♂						0.3	0.4
♀						0	0.1
Banrot® 40% WP Fungicide							
♂	0	0.4	0.5	0.4	0.4	0.4	0.6
♀	0.1	0.6	0.5	0.6	0.4	0.6	0.5
Positive Control (DNCB)							
♂	0.3	2	3	1.2	0.3	–	–
♀	0.5	2.3	3	1.2	2.3	–	–
Ethanol Control for DNCB							
♂	0	0	0	0	0	–	–
♀	0	0	0	0	0	–	–

In this study, the elevated mean irritation scores in control animals (particularly males) resulted in a re–challenge application. However, high irritation scores were also observed in the naïve males used in the re–challenge. This, when coupled with the dampened response of males to the positive control (DNCB) at 48 hours, made it difficult to characterise the severity of Banrot® 40% WP Fungicide's skin sensitising potential. However, since the mean irritation scores for test animals challenged and re–challenged with Banrot® 40% WP Fungicide were essentially the same as that during the induction phase, this effect is considered to be dermal irritation rather than skin sensitisation. Therefore, under the conditions of the study, Banrot® 40% WP Fungicide is not a skin sensitiser in male and female guinea pigs.

Gabriel D (1995a) Acute oral toxicity, Single level – Rats. Gabriel D. Biosearch Incorporated, Philadelphia, Pennsylvania 19134 USA. Test Article S-5096, Batch #5-5005-1CW; T-070; Biosearch project No. 95-8215A. 12/07/95.

Test Compound	ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants (S-5096, Batch #5 5005-1CW; T-070), 25% w/v in deionised water
Test Species	Sprague-Dawley rats, 203 – 263 g from Buckshire Corp., Perkasie, PA 18944
Study Duration	May 1995
GLP& QA:	Yes
Guidelines:	US EPA 81-1 (1984)

Material and methods: Fasted rats, 5/sex, were treated with a single oral dose of ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants at 5000 mg/kg bw by gavage. Clinical signs and mortality were observed daily for a period of 14 days. Bodyweights were recorded weekly. Necropsies and gross pathology examination were performed on all animals sacrificed by carbon dioxide on day 15.

Results: All rats appeared to be normal within 4 hours post dosing. One female was found dead on the next day, and necropsy revealed green anogenital staining and green liquid in the stomach and the lower gastrointestinal tract. All survivors exhibited light green anogenital staining and ruffled fur during days 1 to 5, but became normal from day 6. All rats had expected bodyweight increase during the study. The oral LD_{50} was greater than 5000 mg/kg bw/day.

Gabriel D (1995b) Acute dermal toxicity, Single level – Rabbits. Biosearch Incorporated, Philadelphia, Pennsylvania 19134 USA. Test Article S-5096, Batch #5-5005-1CW; T-070; Biosearch project No. 95-8215A. 12/07/95.

Test Compound	ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants (S-5096, Batch #5-5005-1CW; T-070), moistening with saline
Test Species	New Zealand White rabbits, 2.52-2.99 kg from Davidson Mill Farm, Jamesburg, NJ
Study Duration	April 1995
GLP& QA	Yes
Guidelines	USEPA 81-2 (1984)

Materials and methods: Rabbits, 5/sex, received a single application of ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants at 2000 mg/kg bw to a skin area (10% body surface) on the back for 24 hours under a dressing, followed by gentle wiping with deionised water. Clinical signs and mortality were observed daily for a period of 14 days. Dermal irritation was scored daily using the Draize method. Bodyweights were recorded weekly. Necropsies and gross pathology examination were performed on all animals euthanized using sodium pentobarbital on day 15.

Results: Well defined to severe erythema (score 2 to 4) was observed at the application site of all animals, accompanied by very slight to slight oedema (score 1 to 2). By day 14, erythema was reduced but not completely in 6/10 animals (score 1 to 2, dry, flaky skin or fissure). One female lost weight (90 g) in week 1. One male had no faeces on days 9, 12 and 13, appeared thin and was found dead on day 14. Faecal staining, no formed faeces, greenish fluid in the

stomach and gastrointestinal tract were revealed in this rat by gross pathology. Other animals appeared to be normal during the study and at necropsy. The dermal LD_{50} was greater than 2000 mg/kg bw/day.

Hershman RJ (1995) Acute inhalational toxicity, Single level – 4 hour exposure - rats. Biosearch Incorporated, Philadelphia, Pennsylvania 19134 USA. Test Article S-5096, Batch #5-5005-1CW; T-070; Biosearch project No. 95-8229A. 11/09/95.

Test Compound	ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants (S-5096, Batch #5-5005-1CW; T-070)
Test Species	Sprague-Dawley rats, 226-297 g from Buckshire Corp., Perkasie, PA 18944
Study Duration	August 1995
GLP& QA	Yes
Guidelines	US EPA 81-3 (1984)

Materials and methods: Rats, 5/sex, were placed inside a exposure chamber with an aerosol of 2427 mg/m^3 of ZYBAN WSB Broad Spectrum for Ornamental Plants for 4 hours, followed by air clean of the cage and water-rinse of the body. Particles of the test substance collected from the chamber constituted only 11.3% < 2.1 μm, and 35-53% > 9 μm. Mortality and clinical signs were recorded during and after the exposure. Rats were weighed on days 0, 7 and 14. Gross pathological examinations were performed at necropsy on day 14.

Results: There were no deaths. Animals showed closed eyes and inactivity during exposure. All rats exhibited green-wet staining on eyes, noses and mouths, and were damp and ruffled during the first 2 days after exposure. One female gasped for 2 hours post-exposure, was cold to the touch, showed wet staining of nasal, buccal and anogenital areas, wheezing in the morning, ruffled and a brown-stained muzzle, and audible breathing within 6 days. All signs disappeared from day 7. This and another female had weight loss (6 and 20 g respectively) during week 1. All rats appeared normal during days 7-14. No abnormal changes were seen at necropsy. The inhalational LC_{50} was greater than 2427 mg/m^3.

Comment: Particle size distribution was not satisfactory. The study author claimed that despite the rigorous attempt to reduce the size of particles, 35-53% particles were greater than 9 μm, and only approximately 10% of the test substance remained airborne.

Moore GE (1995a) Primary skin irritation – Rabbits. Biosearch Incorporated, Philadelphia, Pennsylvania 19134 USA. Test Article S-5096, Batch #5-5005-1CW; T-070; Biosearch project No. 95-8215A. 10/07/95.

Test Compound	ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants (S-5096, Batch #5-5005-1CW; T-070), moistening with saline
Test Species	New Zealand White rabbits from Davidson Mill Farm, Jamesburg, NJ
Study Duration	April 1995
GLP& QA	*Yes*
Guidelines	US EPA 81-5 (1984)

Materials and methods: Six rabbits (3/sex) were given a single dermal application of 500 mg (moistened with 0.5 mL saline) of ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants to the pre-clipped intact skin on the back for 4 hours under a semi-occlusive dressing. An adjacent area of untreated skin served as control. The application sites were scored within 30-60 min, at 24, 48 and 72 hours after exposure, according to the Draize Scale.

Results: No signs of skin irritation were observed at the application site of any rabbit at any time. The test substance was not a skin irritant in rabbits.

Comment: Moderate skin irritation was induced by longer exposure (24 hours) at a higher dose (2000 mg/kg bw) in the dermal toxicity study (Study 2), or repeated induction application in the skin sensitisation study (Study 6).

Moore GE (1995b) Primary eye irritation – Rabbits.. Biosearch Incorporated, Philadelphia, Pennsylvania 19134 USA. Test Article S-5096, Batch #5-5005-1CW; T-070; Biosearch project No. 95-8215A. 11/07/95.

Test Compound	ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants (S-5096, Batch #5-5005-1CW; T-070), moistening with saline
Test Species	New Zealand White rabbits from Davidson Mill Farm, Jamesburg, NJ
Study Duration	April 1995
GLP& QA	Yes
Guidelines	USEPA 81-4(1984)

Materials and methods: Six rabbits (4 males and 2 females) were subjected to a single application of 50 mg of ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants to the conjunctival sac of one eye. The other eye remained untreated as control. Eye reactions were assessed at 1, 24, 48 and 72 hours, and 4 and 7 days following instillation, and scored according to the Draize Scale. The eyes were also examined with fluorescein dye at 24 hours.

Results: Slight to moderate conjunctival irritation (redness, chemosis and discharge, score 1 to 2) occurred in all treated eyes after application, in 5/6 eyes at 24 hours, in 3/6 eyes at 72 hours, and completely disappeared on day 7. Slight corneal opacity was observed in 2/6 animals at 24 hours only.

Average ocular irritation scores (n = 6)

1 h	24 h	48 h	72 h	4 day	7 day
7.7	9.2	5.0	3.0	2.7	0.0

The test substance was a moderate irritant to rabbit eyes.

Moore GE (1995c) Guinea pig dermal sensitisation, 9 induction applications – Modified Buehler method.. Biosearch Incorporated, Philadelphia, Pennsylvania 19134 USA. Test Article S-5096, Batch #5-5005-1CW; T-070; Biosearch project No. 95-8215A. 10/07/95.

Test Compound	ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants (S-5096, Batch #5-5005-1CW; T-070), 50% w/v solution in deionised water
Test Species:	Male Hartley guinea pigs, 332-431 g, from Davidson Mill Farm, Janesburg, NJ
Study Duration:	April – May 1995
GLP& QA:	Yes
Guidelines:	US EPA 81-6 (1984)

Materials and methods: The sensitising potential of ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants was determined using a modified Buehler method. For induction, 10 male guinea pigs in the test group were treated with 400 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 400 mg of the test substance was applied to the right flank of the animals for 6 hours. Another group of 10 naïve animals remained untreated during the induction phase but received the same challenge application. 1-Chloro-2,4-dinitrobenzene 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 Buehler sensitisation scoring scale. Bodyweights were recorded pre application and at termination (day 38).

Results: For the test group, increased dermal irritation was observed at the application site during the induction period, i.e. no skin irritation for the first 2 applications, progressing from slight to severe erythema, with or without oedema, dry and flaky skin for the rest of applications. However, except for slight erythema in 1 animal, no dermal responses occurred in the test group after the challenge, which was similar to the naïve control group. In contrast, very faint to moderate erythema was seen in 7/10 animals in the positive control group. ZYBAN WSB Broad Spectrum Fungicide for Ornamental Plants was not a skin sensitiser in this test system.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1 Dermal Application

Naas D J (1991) 21-Day dermal study in rabbits with thiophanate-methyl technical. Laboratory: not specified. Report No: Doc. 532-001, RD-9160. Study duration: 26 June to 17 July 1990. Report date: 1991. Guidelines: OECD 410. GLP: Yes.

Methods: New Zealand White rabbits (5/sex/group, 15 weeks old; 2.1-2.5 kg bodyweight) were dosed with thiophanate-methyl (purity not specified) moistened with water and applied to the shaved intact dorsal skin at 0 (water only), 100 , 300 or 1000 mg/kg bw/d for 21 days (5 applications/week, 1 application/d, 6 h/d, under occlusive condition), Approximately 6%, 3%, 6% or 8% of the total body surface were covered during administration for 0, 100 , 300 or 1000 mg/kg bw/d groups. Mortalities, clinical signs, dermal irritation and food consumption were recorded daily. Bodyweight was recorded weekly. Blood was taken for haematology and clinical chemistry tests at the end of the study. Urinalysis was not performed. Complete necropsy was performed on all rabbits. Brain, kidneys, liver, ovaries and testes were weighted. A microscopic examination was conducted on selected guideline tissues: kidneys, liver, skin (treated and un-treated) and gross lesions.

Results: No dose-related effects were observed at any dose level on survival, clinical signs, haematology and clinical chemistry parameters, bodyweight and organ weight although food consumption was significantly decreased (by 20-30% compared to control group) at high dose. Microscopic findings were limited to the treated skin. Hyperkeratosis of the treated skin was observed in one female in each of the treated groups. Non suppurative subdermal inflammation was also present in 300 mg/kg bw/d female that has hyperkeratosis.

Dermal irritation was limited to sporadic occurrence of very slight erythema and desquamation in all treated groups, generally during the second week of administration. Oedema was not present in any treated group.

Conclusion The NOEL for systemic toxicity was 300 mg/kg bw/d based on decreased food consumption at next higher dose level of 1000 mg/kg bw/d. The NOEL for local toxicity was between 0 to <100 mg/kg bw/d based on hyperkeratosis of the treated skin (one female on each treated group) including the lowest dose level of 100 mg/kg bw/d.

4.2 Oral Dosing

Noguchi T (1970) Studies on the subchronic toxicity of thiophanate-methyl in mice. Nisso Institute for Life Science, Kanagawa, Japan. Report No:RD-73053. Report date: August 1970. Guidelines: Not stated. GLP: Not stated.

Materials and method: SPFCR mice (12/sex/group) received diets containing thiophanate-methyl (purity not stated) at a concentration of 0, 12.8, 64, 320, 1600 or 8000 ppm for 6 months (equivalent to 0, 2/2, 10/11, 50/52, 250/231 or 1240/1630 kg/kg bw/d for M/F, respectively). Homogeneity, stability, and concentrations of the diet and clinical signs of toxicity were also not reported. Mortality, bodyweight, food consumption were observed daily. Blood was taken from all animals for haematology at week 13 and for clinical chemistry at the end of the study after fasting. Urinalysis was performed at week 12. At the

end of the study, all animals were fasted and subsequently sacrificed, and subjected to gross and microscopic examinations. The organ weight of brain, heart, testes, liver, lung, spleen, adrenal, kidney, ovary, thyroid, pituitary and thymus were determined and histopathology was performed.

Results: There were no treatment-related deaths. The body-weight gains were reduced in both sexes at 8000 ppm (25% and 40% lower for males and females, respectively). Erythrocyte counts were reduced in females at 8000 ppm (22% lower) and hematocrit values were reduced in both sexes at 1600 and 8000 ppm (7-12% lower). No significant differences were seen in the weights of the cerebrum, heart, lung, spleen, kidney, or testis, but relative liver weights were increased at 8000 ppm (31% and 43% higher for males and females, respectively). Histopathological investigation revealed a higher incidence of large swollen hepatic cells, some with oedematous or granular protoplasm, with five cases in males and three in females at 8000 ppm and one case in each sex at 1600 ppm.

Conclusion: The NOEL was 320 ppm, equivalent to approximately 50 mg/kg bw/d, on the basis of hepatotoxic and haematological effects at 1600 and 8000 ppm.

Nishibe T and Takaori H (1990). Thiophanate-methyl: Subchronic study in rats. Environmental Toxicology Laboratory, Kanagawa, Japan. Report No: 0565. Report date: 25 June 1990. Guidelines: OECD 408. GLP: Yes.

Materials and methods: Fisher 344 rats (10/sex/group, 6 weeks old, bw: 93.9-115 g) received diets containing thiophanate-methyl (purity 96.55%) at a concentration of 0, 200, 2200, 4200, 6200 or 8200 ppm (equivalent to 0, 14/16, 155/173, 293/323, 427/479, 565/647 kg/kg bw/d for M/F, respectively) for 13 weeks. Homogeneity and concentration analysis revealed that the target doses were achieved. Signs of clinical toxicology, mortality, bodyweight, food consumption were observed daily. Ophthalmoscopic examinations were carried out on all animals of control and high dose groups prior to study and at week 12. Blood was taken from all animals for haematology at week 13 and for clinical chemistry at the end of the study after fasting. Urinalysis was performed at week 12. At the end of the study, all animals were fasted and subsequently sacrificed, and subjected to gross and microscopic examinations. The organ weight of brain, heart, testes, liver, lung, spleen, adrenal, kidney, ovary, thyroid, pituitary and thymus were determined and histopathology was performed.

Observations: There no were no deaths or clinical signs. Bodyweight changes and food were not affected by treatment. No ophthalmoscopic abnormalities were observed.

Haematology and clinical chemistry: There were changes in haematology and clinical chemistry in both sexes (Table 8). It should be noted that most of these changes were slight in magnitude and/or were within the historical control range for F344 rats at similar ages and/or without an apparent dose-response pattern. Thus, most of these haematological and clinical chemistry changes were not considered toxicologically significant except for cholesterol and thyroxine levels. Total cholesterol levels were increased in both sexes at 2200 ppm and above while T3 levels were increased in both sexes at 8222 ppm. Urinalysis did not reveal any significant findings.

Table 8: Significant changes in haematology and clinical chemistry (doses in ppm)

Parameter	Male						Female					
	0	200	2200	4200	6200	8222	0	200	2200	4200	6200	8222
Haematology (n=10)												
RBC ($10^6/m^3$)	8.1	8.1	8.06	8.05	7.96	7.9	7.63	7.54	7.55	7.40	7.43	7.44
Ht (%)	47.5	47.3	46.3	46.2	46*	45**	47.7	47.2	46.4	45.2**	45**	45.1**
Hb (g/dl)	15.2	15	14.6*	14.5**	14.3**	13.9**	15.3	15	14.7*	14.2**	14.2**	14.1**
MCV (μg^3)	58.6	58.5	57.5*	57.4**	57.8**	57.6*	62.5	62.6	61.4	61.1**	60.6**	60.6**
MCH (µg)	18.7	18.6	18.2**	18**	18**	17.7**	20.1	20	19.5**	19.2**	19.1**	19**
MCHC (%)	31.9	31.8	31.3	31.2**	31.3**	30.9**	32.2	31.9	31.8**	31.5**	31.6**	41.4**
Clinical chemistry (n=10)												
Cholesterol (mg/dl)	46.5	48.8	75.4*	82.2**	91.2**	106.4**	66.1	63.2	94.2**	108**	124**	143**
Protein (g/dl)	6.4	6.3	6.9*	7.1**	7.1**	7.2**	5.9	5.9	6.4**	6.6**	6.8**	7**
Albumin (g/dl)	4	4.3	4.4**	4.4**	4.4**	4.5**	3.9	3.9	4.2*	4.3**	4.3**	4.4**
Calcium (mg/dl)	9.4	9.3	9.8*	9.9**	10**	9.9**	9.2	9.2	9.3	9.4	9.4	9.5
Ch-E (IU/L)	464	469	506	564**	586**	639**	3441	3050	2844**	2596**	2606**	2490**
T3 (ng/mL)	0.7	0.73	0.76	No data	No data	0.96**	0.69	0.73	0.81	No data	No data	1.08*

*p<0.05; **p<0.01

Gross examination and histopathology: Dose-related increases of liver and thyroid weights were observed in both sexes at 2200 ppm and above (Table 9). Gross inspection revealed swelling of the thyroid gland in males at 4200 ppm and above (1/10, 2/10 and 6/10, respectively) and in females at 6200 and 8200 ppm (2/10 and 4/10, respectively). Histopathological examinations revealed increased incidence of follicular hyperplasia/hypertrophy in thyroids and hepatocellular swelling/lipofuscin in the liver of both sexes in all animals at 2200 ppm and above.

Table 9: Significant changes in organ weights (doses in ppm)

Parameter	Male						Female					
	0	200	2200	4200	6200	8222	0	200	2200	4200	6200	8222
Bodyweight (g)	314	319	313	317	315	319	174	177	173	166	173	177
Thyroid												
Absolute (mg)	23	24	32**	44**	55**	66**	16	20	24**	27**	37**	39**
Relative (x1000)	0.07	0.07	0.1**	0.14**	0.17**	0.2**	0.09	0.1	0.14**	0.16**	0.21**	0.22
Liver												
Absolute (g)	7.8	8.5	9.9**	10.8**	11.8**	12.7**	4.3	4.4	5.2**	5.6**	6.6**	7.1**
Relative (x100)	2.5	2.66	3.1**	3.4**	3.7**	4.0**	2.4	2.5	3.0**	3.4**	3.8**	4.0

*p<0.05; **p<0.01

Conclusion: The NOEL was 200 ppm (14 mg/kg bw/d) based on changes in total cholesterol and thyroxine levels, thyroid and liver weights and histopathological changes in the thyroid and liver at 2200 ppm and above.

Auletta CS (1991) Thiophanate-methyl: Subchronic (3 months) oral toxicity in dogs. Biodynamics Inc. New Jersey, USA. Report No: Doc. 89-3525. Study duration: 14 February – 23 May 1990. Report date: 10 May 1991. Guidelines: OECD 409. GLP: Yes.

Materials and methods: Thiophanate-methyl (purity 96.55%) was orally administered, in gelatine capsules, to Beagle dogs (4/sex/group, 7 months old, bw: 6.1-9.7 kg) at dose levels of 50, 200 or 800 mg/kg bw/d for 90 days. However, severe toxicity was noted at 800 mg/kg bw/d hence this high dose was reduced to 400 mg/kg bw/d on day 50. Animals were observed for signs of clinical toxicity and mortality twice a day and bodyweight and food consumption were observed weekly. Ophthalmoscopic examinations were carried out on all animals of control and high dose groups prior to study, then weekly and at terminal sacrifice. Blood was taken from all animals for haematology and clinical chemistry, and urine for urinalysis before commencement and at 1.5 and 3 month after fasting. All animals were sacrificed at the end of study, and all animals died during the study had complete gross examinations. Organ weights and histopathology were carried out on the full set of guideline organs.

Observations: No deaths were observed, except 1 animal at 800 mg/kg bw/d was euthanased as moribund on day 41. All animals at the highest dose appeared thin and/or dehydrated throughout the treatment period. Compared to controls, a dose-related decrease in bodyweight was observed at 200 and 800 mg/kg bw/d in both sexes (13-17% lower). This was accompanied by reduced food consumption in both sexes (24-35% lower). One male at the highest dose had a 49% loss in bodyweight which caused moribund condition during week 7.

Haematology and clinical chemistry: Decreased RBC, haemoglobin, hematocrit and increased activated thromboplastin times were observed at the highest dose. Decreases in T3 levels were observed in both sexes at 200 and 800 mg/kg bw/d, but no changes in TSH. No abnormalities were found in ophthalmoscopic examination and urinalysis (Table 10).

Organ weight and pathology: Relative liver weights were increased in both sexes at 200 mg/kg bw/d and above. Relative thyroid weights were seen in males at 200 mg/kg bw/d and above. Gross post-mortem examinations revealed emaciation, a finding consistent with bodyweight loss, in 1 mid-dose and 3 high dose males. Microscopic examination revealed dose-related hypertrophy and hyperplasia of the follicular epithelial cells of the thyroid at 200 mg/kg bw/d. The severity of hypertrophy and hyperplasia was more pronounced at the highest dose.

Conclusion: No NOEL was established based on the finding that one animal in each sex had follicular cell hypertrophy in the thyroid at 50 mg/kg bw/d, the lowest dose tested.

Table 10: Significant findings (doses in mg/kg bw/d)

Parameter	Male				Female			
	0	50	200	800	0	50	200	800
Haematology (n=4)								
RBC (mil/µl)								
Pre-treatment	6.56	6.66	6.36	6.0	6.54	6.53	6.72	6.78
Week 6	6.7	6.85	6.42	5.74	6.76	7.04	6.67	6.06
Week 13	7.17	6.60	6.4	5.5*	7.0	7.01	6.05*	5.85**
Hb(g/dl)								
Pre-treatment	16.8	16.7	16.5	15.7	16.4	16.4	17.1	17.41
Week 6	17	17	16.2	14.5	17.1	17.5	17.1	15.7**
Week 13	18	16.7	15.9	13.6*	17.4	17.4	15.5*	15.3**
Ht (%)								
Pre-treatment	48	47	46	44	47	46	49	49
Week 6	46	47	44	40	47	48	48	44
Week 13	51	47	45	39*	49	49	44	44*
APTT (sec)								
Pre-treatment	9.6	9.7	9.2	10.2	9.5	9.9	9.6	10
Week 6	9.3	9.2	9.2	10.4**	9.5	9.7	9.7	10.3*
Week 13	9.2	9.2	9.1	10.2**	9.2	9.6	9.6	9.9*
Clinical chemistry (n=4)								
T3 (ng/mL)								
Pre-treatment	1.35	1.36	1.37	1.35	1.3	1.35	1.26	1.27
Week 13	1.38	1.51	1.21*	1.08**	1.8	1.55	1.34**	1.35**
Organ weights (n=4)								
Bodyweight (kg)	10.4	9.3	8.7*	7.5**	7.5	8.1	6.2*	5.9**
Thyroid								
Absolute (g)	0.7	0.76	1.0	1.15	0.77	0.76	1.0	1.15
Relative (x10000)	0.75	0.82	1.15**	1.55**	1.0	0.94	1.2	1.2
Liver								
Absolute (g)	313	296	332	321	264	286	2.93	281
Relative (x1000)	3.0	3.2	3.8**	4.3**	3.57	3.56	4.73**	4.78**
Histology (n=4)								
Thyroid								
Follicular cell hypertrophy	0	1	3	4	0	1	2	4
Follicular cell hyperplasia	0	0	1	4	0	0	0	3

*p <0.05, ** p <0.01

5. CHRONIC/CARCINOGENICITY STUDIES

Tompkin CE (1993) Thiophanate-methyl – 18-month dietary study in mice. Nippon Soda Co., Ltd. Tokyo, Japan. Study number: RD-9328 (unpublished) Report date: 27 August 1993.

Test chemical:	Thiophanate-methyl (purity 96.5%)
Test species:	Mice (Crl:CD-1), 4 w.o, 24.8-33.1 g (m) and 19.6-25.8 g (f) from Charles River Breeding Laboratories, Porgate, Michigan, USA
GLP and QA:	Yes
Guidelines:	EPA 83-2

Materials and methods: Mice (60/sex/dose) received thiophanate-methyl at 0, 150, 640, 3000 or 7000 ppm in the diet for weeks for 78 weeks. Average daily intake values of the test chemical for males and females were 0/0, 24/29, 99/123, 467/558 or 1079/1329 mg/kg bw/d respectively. Stability, concentration and homogeneity of thiophanate-methyl in the diet were analysed and proved to be satisfactory. The animals were observed daily for mortality and clinical signs. Physical examination was performed weekly for the main group, while food consumption and bodyweight were recorded regularly. All surviving animals were necropsied at week 39 for the satellite group (10/sex/dose) or at week 78 for the main group (all animals). Samples were taken for haematological examinations, organs were weighed and tissues examined microscopically.

Observations: By the end of the study, cumulative mortality rates for males and females were 17 (control), 18, 23 and 27% and 20 (control), 22, 25 and 38%, respectively. Amyloidosis and atrial thrombosis were listed as the main cause of death. No treatment-related clinical signs were apparent. Palpable mass data were comparable among groups. Compared to control group, bodyweight gains and food consumption were not significantly affected in treated groups. Haematological changes were not remarkable.

Pathology: At week 39, 3/10 males had enlarged thyroid glands at 7000 ppm. In the satellite group, relative liver weight was higher in both sexes at 3000 and 7000 ppm in the satellite group (6.3** and 7.1** versus 5.0 respectively for males and 6.4** and 8.1** versus 5.0 respectively for females). In the main group, relative liver weight was higher in both sexes at 7000 ppm in the satellite group (10.5** versus 5.6 for males and 7.9** versus 6.1 for females) (Table 11).

Table 11: Significant organ weight and histopathological findings in liver (doses in ppm)

Parameter	Male					Female				
	0	150	640	3000	7000	0	150	640	3000	7000
Satellite group at week 39 (n=10)										
Hepatocellular hypertrophy	5	3	6	10	10	0	1	5	6	10
Main group at week 78 (%)										
Hepatocellular hypertrophy	2.5	6	6	10	30	0	1	0	0	0
Hepatocellular adenoma (n=50)	8	16	14	38	48	0	0	6	16	36

Significantly increased incidences of hepatocellular hypertrophy were observed in males at weeks 39 and 78 at 3000 and 7000 ppm but only slightly so in the females at week 78 at 7000

ppm. At week 78, increased incidences of liver adenoma were observed in males at 3000 and 7000 ppm and in females at 640 ppm and above. This was based on the historical control range of liver adenoma compiled by Charles River Laboratories for their CD-1 mice on 18-month carcinogenicity studies from 1978-1984 (0-16% for males and 0-2.7% for females). Twenty five percent of those studies had an incidence of 16% for males. It is not clear why hepatocellular hypertrophy was not detected in females at any dose at week 78. Only two hepatocellular carcinomas were seen: one male each at 640 and 7000 ppm (historical control range for liver carcinomas for CD-1 males is 0-6%). Other neoplastic lesions were isolated cases with random distribution in groups and thus were not considered to be treatment-related.

The NOEL was 640 ppm (99 mg/kg bw/d) for males and 150 ppm (29 mg/kg bw/d) for female rats based on increased liver weight, hepatocellular hypertrophy and adenoma at higher doses (males) and on a higher incidence of benign hepatocellular adenoma at 640 ppm and above (females).

Hashimoto Y and Tsubura Y (1972). Final Report on the Long-term Oral Toxicity Studies of Thiophanate-methyl, Demethyl 4,4'-o-phenylenebis (3-thioallophanate) in Rats of Sprague Dawley Strain for 24 Months. Nisso Institute for Life Science, Nippon Soda Co., Ltd, Japan. IIA 5.5.1/02 Doc No. 537-004; RD-73057.

Test chemical:	Thiophanate-methyl, tech. (>94 % purity)
Batch No:	NA
Test species:	Rats (Sprague Dawley)
Duration of study:	24 months
GLP and QA:	QA, pre-GLP
Guidelines:	NA

Material and methods: Sprague Dawley Rats (35/sex/dose, 50/sex for control group) received thiophanate-methyl (purity >94%) via the diet at 0, 10, 40, 160 and 640 ppm (equivalent to 0, 0.5/0.5, 2/2, 8/8 and 30/34 mg/kg bw/d for M/F, respectively) for 24 months. After 3 and 12 months, 5/sex from each group and, at the end of the study, all surviving animals, were sacrificed and complete necropsies performed.

Clinical signs and mortalities were monitored daily, bodyweight monitored weekly, and food consumption monitored every 10 days, on each rat at all dose groups. Haematology and urinalysis were carried out on for 5 rats of each sex from each dose group at months 3, 6, 12, 18 and 24, and clinical chemistry including serum alkaline phosphatase, serum glutamic oxalic transaminase, glucose, total serum protein, albumin and protein bound iodine were obtained for 5 rats of each sex from each dose group at months 3, 12 and 24. All animals at terminal sacrifice were assessed by gross pathology. The following organs from each rat were weighed and organ/terminal bodyweight ratios calculated; brain, heart, lung, liver, spleen, thymus, kidneys, adrenals, thyroid, hypophysis and gonads (testis and ovaries). Histopathological examination was carried out on the following tissues; cerebrum, cerebellum, thyroid, parathyroid, heart, lung, liver, spleen, stomach, colon, small intestine, adrenal gland, genital organ, thymus, hypophysis, uterus, bone marrow, breast, kidney, urinary bladder, submaxillary gland, parotid gland, lymph node, aorta and femoral muscle.

Results:
Observations: There was no dose related mortality in rats treated with thiophanate methyl. By the end of the study, cumulative mortality rates for males and females were 57.5 (control), 68, 64, 60 and 68% and 50 (control), 72, 52, 44 and 40%, respectively. The mortality in control groups was slightly lower than some thiophanate-methyl groups it was not dose-related. The causes of deaths included broncho pneumonia, nephrosis of the kidney, spontaneous hypophysis adenoma, spontaneous fibroadenoma of breast and otitis mycotica and incidence of these deaths in rats across all dose groups including controls was not significantly different.

No clinical signs of intoxication were observed among rats of both sexes in any dose group. There was a slight but significant decrease in bodyweight gain for high dose (640 ppm) males and females in comparison to controls (by 13%/16% in M/F, respectively). Apart from a slight decrease in food consumption of females in the high dose group (640 ppm) at 18 and 24 months, food consumptions were comparable to those of controls.

Haematology, clinical chemistry and urinalysis: The haematological findings of both sexes from all dose groups showed the values to be within normal limits and comparable to their controls. No treatment related changes were noted in urinalysis or in clinical blood chemistry. It should be noted that thyroid hormones were not measured in clinical blood chemistry.

Gross pathology: Macroscopic observations of testicular atrophy were observed at terminal necropsy, one in each of the control, 10 ppm and 40 ppm groups, two in the 160 ppm group and five in the 640 ppm group (table 30). This effect was considered to be treatment related in the high dose group only (640 ppm) due to associated treatment related histopathological observations of effects on spermatogenesis in three animals. Treatment related macroscopic changes were not observed in any other tissues or organs.

Organ weight: No treatment related absolute or relative organ weight changes were observed.

Histopathology: Treatment related histopathological findings were observed only in 640 ppm group. In males, an increase in hypertrophy of follicular epithelium, a decrease of colloidal substance in thyroid was observed. Histopathological observations of effects on spermatogenesis were noted in six males at 640 ppm, (two males at 160 ppm and one each in other groups including the control), with three of the animals in the high dose group showing associated observations of testicular atrophy at terminal necropsy (Table 12).

The effects on spermatogenesis in one animal in each of the 10 and 40 ppm, and two animals in the 160 ppm dose groups are considered to be sporadic and not treatment related. This is consistent with hypospermatogenesis observed in one of the control animals. However, the effects on spermatogenesis in the 640 ppm dose group (6/35 animals) are considered to be treatment related. This is considered an appropriate conservative measure as macroscopic observations of testicular atrophy were observed in three of the six animals and histopathological observations of effects on spermatogenesis have been obtained in rats treated with the ethyl derivative of thiophanate at a similar dose (31.25 mg/kg bw/d). Additionally, a critical endpoint of toxicity for the metabolite carbendazim is testicular effects.

No other treatment related histopathological effects were noted. In particular, a treatment related increase in the incidence of neoplasia was not noted.

Table 12: Macroscopic and microscopic histopathological findings in testis (doses in ppm)

Dose (number of animals in group)	Animal identification number	Number of weeks in which animal (Died/Killed)	Histopathological examination (testicular effects)	Observations of testicular effects at terminal necropsy
Control (50)	6013	103 (killed)	2TS	atrophy of right testis
10 ppm (35)	6414	83 (died)	none	atrophy of testis
	6434	103 (killed)	3TS	none
40 ppm (35)	6321	88 (died)	none	atrophy of testis
	6322	103 (killed)	3TS	none
	6331	103 (killed)	moderate congestion – atrophy of testis	none
160 ppm (35)	6216	94 (died)	2TS	none
	6217	59 (died)	none	atrophy of testis
	6230	103 (killed)	2TS	atrophy of testis
640 ppm (35)	6118	90 (died)	none	atrophy of testis
	6119	87 (died)	none	atrophy of testis
	6122	103 (killed)	2TS	none
	6123	103 (killed)	1TS	none
	6124	103 (killed)	2TS	atrophy of testis
	6125	103 (killed)	2TS	none
	6130	91 (died)	2TS	atrophy of testis
	6132	103 (killed)	2TS	atrophy of testis

1TS = diminution of spermatogenesis, 2TS = hypospermatogenesis, 3TS = aspermatogenesis

Conclusion: The NOEL was 160 ppm (8 mg/kg bw/d) based on reduced bodyweight gains and, histopathological changes in the thyroid and effects on spermatogenesis at the highest dose.

Takaori H (1993) Combined long-term (2-years) oral toxicity and carcinogenicity in the rat. Laboratory: not specified. Report No: Doc. 537-001, RD-9327.

Test chemical: Thiophanate-methyl, tech. (96.55 % purity)
Batch No: TIF-1016
Test species: Rats (Fisher 344), 60/sex/dose, 6 w.o., 121.1-121.2 g ±6.0-6.1g (male), 99.7 g±3.1-3.2g (female) from Charles River Japan, Atsugi, Kanagawa
Duration of study: 27 November 1990-4 December 1992
GLP and QA: Yes
Guidelines: OECD 453

Materials and methods: Rats (60/sex/dose) received thiophanate-methyl orally by gavage at 0, 75, 200, 1200 or 6000 ppm (equivalent to 0, 3/4, 9/10, 54/63 or 280/334 mg/kg bw/d for M/F, respectively) for 24 months. All animals were observed daily for mortality and clinical signs, while food and water consumption and bodyweight were recorded weekly for the first 14 weeks and every forth week. Ophthalmological examinations were carried out prior to the start and at 6, 12, 18 and 24 months. Haematology was tested at 3, 6, 12, 18 and 24 months,

clinical chemistry (including T3, T4 and TSH) and urinalysis were tested at 6, 12, 18 and 24 months. Necropsy was performed on 10/sex/dose (except 5 males at 6000ppm group) at week 52, all surviving animals at the end of the treatment, and all rats that died or were sacrificed during the course of the study. Eleven organs (brain, liver, adrenal, heart, lung, kidney, testis, spleen, ovary, thyroid and pituitary) were weighed and tissues examined microscopically on a full set of guideline organs.

Observations: By the end of the study, cumulative mortality rates for males and females were 26 (control), 30, 48, 42 and 96% and 26 (control), 24, 16, 24 and 22%, respectively. Mortality in males at 6000 ppm was significantly higher than control, with only two males survived at the end of the study (eight males were killed due to technical reasons). The main causes of death were nephropathy (22 rats), thyroid adenoma (12 rats) and leukaemia (six rats). There were no treatment-related clinical signs. Reduced bodyweight gains were observed in both sexes at 1200 and 6000 ppm (21% and 38% lower for males and 13% and 32% for females, respectively). Food consumption was not affected by treatment. No ophthalmoscopic abnormalities were observed at all test times.

Haematology, clinical chemistry and urinalysis: Treatment-related anaemia (decreases of RBC, Hb, hematocrit, MCV, MCH and MCHC) was noted in both sexes of 6000 ppm group at months 3, 6, 12, and 19. Increased platelet counts (both sexes) and white blood cell counts (male) were noted in the 6000 ppm group (Table 13 & 14).

Table 13: Significant haematological findings in males (n= 10)

Study month	Dose (ppm)	RBC (10^6/mm^3)	PCV (%)	Hb (g/dl)	MCV (μ^3)	MCH (pg)	MCHC (%)	Platelet (10^6/mm^3)	WBC (10^3/mm^3)
3	0	8.05	47.9	15.0	59.5	18.6	31.3	.697	6.5
	200	8.04	47.9	14.9	59.6	18.6	31.2	.674	6.2
	1200	7.93	46.7	14.5	58.9	18.3	31.1	.706	5.9
	6000	7.87	45.8*	14.1**	58.2*	17.9**	30.8*	.734*	6.1
6	0	8.19	48.7	15.5	59.4	18.9	31.9	.644	6.5
	200	8.35	50.0	15.7	59.9	18.8	31.4	.645	6.5
	1200	8.16	48.0	15.0	58.9	18.4	31.3	.672	6.3
	6000	7.85	46.1*	14.1**	58.8	18.0**	30.6	.732**	7.4*
12	0	8.15	49.3	15.2	60.4	18.6	30.8	.660	5.5
	200	8.00	48.5	15.1	60.7	18.9	31.1	.667	5.2
	1200	7.97	47.9	14.4**	60.1	18.1	30.2	.709	5.6
	6000	7.53**	44.4**	13.3**	59.0*	17.7**	30.0	8.34**	7.1*
18	0	7.50	45.9	14.8	61.2	19.7	32.3	.651	5.2
	200	7.37	45.7	14.5	62.0	19.7	31.7	.650	5.1
	1200	7.15	43.4	13.5	60.7	18.9	31.1	.785	6.5
	6000	6.42**	38.0**	11.7**	59.0	18.1**	30.6*	.968**	8.5*
24	0	6.38	42.2	13.1	68.2	20.8	30.8	.640	13.6
	200	6.38	41.7	13.0	65.5	20.4	31.2	.664	5.5
	1200	5.54	36.6	10.9	67.9	20.1	29.6	.713	13.5
	6000	6.45$^+$	39.6$^+$	12.0$^+$	61.4$^+$	18.6$^+$	30.3$^+$	.659$^+$	7.7$^+$

*p<0.05; ** p<0.01; *** p<0.001; $^+$ Only 1 males at 24 month so statistic analysis was not possible.

Table 14: Significant haematological findings in females (n= 10)

Study month	Dose (ppm)	RBC (10^6/mm^3)	PCV (%)	Hb (g/dl)	MCV (μ^3)	MCH (pg)	MCHC (%)	Platelet (10^6/mm^3)	WBC (10^3/mm^3)
3	0	7.45	46.5	14.9	62.4	20.2	32.1	.693	4.8
	200	7.47	46.2	14.7	62.0	19.8	31.9	.710	5.0
	1200	7.52	46.6	14.7	62.0	19.6*	31.6	.715	4.7
	6000	7.45	45.1	14.2**	60.5**	19.0**	31.4**	.714	4.8
6	0	7.69	49.0	15.5	63.7	20.2	31.8	.669	5.3
	200	7.61	48.2	15.4	63.4	20.2	31.9	.679	5.6
	1200	7.60	48.0	15.4	63.1	20.3	32.2	.702	5.3
	6000	7.52	45.6*	14.4**	60.7**	19.1**	31.5	.757**	5.6
12	0	7.39	47.9	15.1	64.8	20.5	31.7	.621	4.0
	200	7.41	47.6	15.0	64.2	20.3	31.6	.630	3.3
	1200	7.53	47.1	14.8	62.6**	19.7**	31.5	.625	3.4
	6000	7.46	45.4*	14.0**	60.8**	18.7**	30.9	.652	3.2
18	0	7.14	45.4	14.9	63.7	21.0	33.1	.667	2.9
	200	6.99	44.2	14.7	63.2	21.0	33.3	.581	3.0
	1200	6.78	42.5	14.4	62.9	21.4	34.1	.598	3.0
	6000	7.17	43.5	14.2	60.6**	19.8	32.7	.620	3.1
24	0	6.64	43.7	13.9	65.9	21.0	31.9	.683	3.1
	200	6.66	43.1	13.8	64.9	20.7	31.9	.669	4.6
	1200	6.58	43.0	13.6	65.6	20.7	31.5	.679	4.3
	6000	6.37	41.1	12.9	64.6	20.3	31.3	.658	3.8

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

Occasionally statistically significant changes in Hb, MCV and MCH were observed in 1200 ppm group of males and/or females between 3-12 months, but returned to normal range by 18-24 months. Differential WBC counts remained normal at all test times except for 6000 ppm males at month 24 could not be evaluated as there was only one animal left at that time point (Tables 6-1-1 and 6-1-2). Since changes were small, and/or within the historical control ranges, and/or without an apparent dose-related relationship, they were not considered to be toxicologically significant.

Statistically significant increased levels of total cholesterol and total protein, as well as a decrease of A/G ratio were noted in both sexes of 1200 and 6000 ppm groups at months 12 and/or 18. At month 24, statistically significantly increased BUN (75, 200 and 1200 ppm groups) and creatinine (1200 ppm group) were observed in males. Decreased levels of chloride, potassium, and decreased activities of LDH, ALAT, ASAT and CPK were observed predominantly in females of 1200 and 6000 ppm groups at 6 and 12 months. Decreased chloride, ALAT and ASAT were observed in 6000 ppm males after 12 months. It was noted that ChE increased significantly in 6000 ppm males but decreased in 1200 and 6000 ppm females at 6 and 12 months. Apart from the total cholesterol (chol) level, total protein and A/G ratio, all other changes were small in magnitude and were generally within historical control ranges, and/or lack of dose-response, thus they were not considered to be toxicologically significant (Table 15 & 16).

Table 15: Significant blood biochemistry findings in males (n=10)

Blood chemistry	Study month	0 ppm	75 ppm	200 ppm	1200 ppm	6000 ppm
Total Chol (mg/dl)	**6**	80.3	84.3	89.6	100.7	192.8**
	12	111.0	110.3	134.4	142.7	285.0**
	18	159.9	137.9	156.6	195.5	330.5**
	24	239.8	275.1	332.6	389.1*	405.8+
Total Protein (g/dl)	**6**	8.3	8.3	8.4	8.6*	8.7**
	12	7.9	7.8	8.0	7.9	7.9
	18	7.6	7.6	7.7	7.5	7.3
	24	7.4	7.0	7.5	6.7	8.1
A/G Ratio	**6**	1.35	1.33	1.34	1.31	1.12**
	12	1.34	1.37	1.31	1.21	0.92**
	18	1.22	1.16	1.10	1.01	0.78**
	24	1.02	0.86	0.85	0.69**	0.72+
BUN (mg/dl)	**6**	17.9	17.9	17.7	16.3	18.5
	12	21.1	20.3	21.4	21.6	26.5
	18	19.5	19.3	18.2	20.7	44.5**
	24	19.4	32.4**	29.3*	38.6**	51.2+
Creatinine (mg/dl)	**6**	0.77	0.74	0.87	0.83	0.85
	12	0.65	0.65	0.63	0.69	0.73
	18	0.67	0.64	0.78	0.80	1.20*
	24	0.85	1.05	1.07	1.34**	1.42+
Chloride (meq/l)	**6**	106.5	108.3	105.5	105.2	103.6*
	12	105.0	105.9	104.4	104.5	101.8**
	18	104.9	105.9	104.8	104.2	102.5
	24	107.2	105.3	102.7	106.8	115.8
Ch-E (IU/l)	**6**	556	527	595	598	792*
	12	1072	1273	1330	1251	1512**
	18	1302	1027	1214	1344	1524
	24	2280	2141	2662	2072	1413

*p<0.05; **p<0.01; + only 1 animal so statistic analysis was not possible.

Table 16: Significant blood biochemistry findings in females (n=10)

Blood chemistry	Study month	0 ppm	75 ppm	200 ppm	1200 ppm	6000 ppm
Total Chol (mg/dl)	**6**	102.7	103.3	107.9	124.0**	204.2**
	12	129.6	134.6	138.5	170.2**	247.1**
	18	137.6	136.6	131.9	155.3	247.9**
	24	150.8	170.5	160.0	209.8	366.6**
Total Protein (g/dl)	**6**	8.3	8.4	8.4	8.8**	9.2**
	12	8.3	8.2	8.2	8.5	8.8**
	18	8.1	8.1	8.4	8.3	8.5
	24	8.0	8.1	8.0	7.9	8.3
A/G Ratio)	**6**	1.52	1.52	1.48	4.41	1.31**
	12	1.74	1.68	1.65	1.62	1.45**
	18	1.48	1.48	1.32	1.49	1.62
	24	1.42	1.48	1.41	1.27	1.07**
BUN (mg/dl)	**6**	16.7	15.9	15.3	16.2	17.5
	12	16.3	20.3**	17.2	16.3	18.7
	18	13.6	16.1	14.6	14.6	16.4
	24	16.7	16.3	16.4	19.3	18.7
Chloride (meq/l)	**6**	108.4	106.1	106.8	105.9	104.5

Blood chemistry	Study month	0 ppm		75 ppm		200 ppm		1200 ppm		6000 ppm	
	12		104.8		105.3		104.8		103.8		102.8*
	18		103.4		105.1		104.4		102.7		103.3
	24		103.7		102.2		106.4		102.7		100.1
Ch-E (IU/l)	**6**		4071		3922		3639		3247**		2629**
	12		4741		4794		4596		3878**		3793**
	18		3557		3723		3459		2915		3326
	24		3961		4055		3819		3118*		3758

*p<0.05; **p<0.01; + only 1 animal so statistic analysis was not possible.

In 6000 ppm males, T3 and T4 were lower than those of controls. T4 was also lower in 1200 ppm males and 6000 ppm females. TSH was higher at 6000 ppm in both sexes, indicating treatment related effects on thyroid hormone production and homeostasis (Table 17).

Table 17: Significant thyroid hormone findings (n=8)

ppm		Male				Female			
		0	**200**	**1200**	**6000**	**0**	**200**	**1200**	**6000**
T3 (ng/mL)									
Study month	6	0.915	0.905	1.031	0.801	0.975	0.860	0.843	0.894
	12	0.923	0.850	0.916	0.783*	0.828	0.899	0.989	0.993
	18	0.776	0.775	0.764	0.573**	0.785	0.796	0.918	0.845
	24	0.791	0.776	0.600	NE	0.830	0.870	0.949	0.935
T4 (µg/100 mL)									
	6	7.08	6.35	6.40	4.70**	4.65	4.09	4.00	4.61
	12	5.89	5.41	5.61	4.70**	3.94	3.81	4.24	4.84
	18	5.38	5.48	4.93	2.41**	4.70	4.06	4.13	3.41**
	24	2.71	2.46	1.48*	NE	2.84	2.71	3.05	2.34
TSH (ng/100µL)									
	6	0.605	0.733	0.836	1.309**	1.021	1.181	1.076	1.298
	12	0.645	0.791	0.768	1.083**	0.473	0.495	0.509	0.750
	18	0.709	0.870	0.886	1.044***	1.116	1.238	1.266	2.004**
	24	0.774	0.863	0.841	NE	0.595	0.576	0.591	0.813*

*p<0.05; **p<0.01; ***p<0.001. NE: not examined.

Dose-related and statistically significant increases in urinary protein were noted in males of 200, 1200 and 6000 ppm groups from month 6, 12 and 24, respectively (Table 18). Although males at 200 ppm group showed a statistically significant increase in urinary protein at month 24, histopathological examination revealed no renal abnormality at this dose.

Table 18: Urinary Protein in Male Rats (n=10)

	Protein (mg/dl)			
Dose (ppm)	**6 month**	**12 month**	**18 month**	**24 month**
0	92.7	466.6	919.4	1050.6
75	52.1	376.2	878.6	1841.3
200	69.5	467.5	839.0	2256.5*
1200	107.8	1167.2*	1398.2	3380.0**
6000	674.0**	1233.5*	2132.4**	3065.0+

*p<0.05; **p<0.01; + only 1 animal so statistic analysis was not possible.

Organ weight: Increases in the liver, kidney and thyroid relative weights were observed in both sexes of ≥1200 ppm groups at months 12 and 24, as well as in spleen of females at month 24 (Table 19).

Table 19: Significant organ weight findings (n= 5-39)

ppm		12 Month				24 Month			
		0	200	1200	6000	0	200	1200	6000
	Organ weight (g)								
Male	Bodyweight (g)	438.9	453.2	433.2	411.0	417.9	408.3	349.3***	298.8[+]
	Liver								
	Absolute (g)	11.24	12.10	13.29**	17.85***	12.84	13.81		19.14[+]
	Relative (%)	2.56	2.67	3.07	4.34	3.10	3.46	16.02*** 4.63***	6.46[+]
	Kidney (R)								
	Absolute (g)	1.33	1.39	1.41*	1.65***	1.62	1.75	1.94***	1.90[+]
	Relative (%)	.303	.308	.327*	.402***	.391	.443	.571***	.635[+]
	Thyroid								
	Absolute (g)	.024	.029	.033**	.060***	.033	.071	.041***	.320[+]
	Relative (%)	.005	.006	.008**	.015***	.008	.022	.012***	.103[+]
	Spleen								
	Absolute (g)	.678	.702	.684	.713	1.88	1.19	2.35	9.71[+]
	Relative (%)	.155	.155	.158	.174	.455	.296	.683**	3.391[+]
Female	Bodyweight (g)	249.6	245.6	228.8	217.4	310.9	299.1	281.9	238.0***
	Liver								
	Absolute (g)	5.52	6.04	6.40*	8.12**	7.69	8.27	9.55***	10.81***
	Relative (%)	2.20	2.46	2.82**	3.75**	2.49	2.78	3.44***	4.60***
	Kidney (R)								
	Absolute (g)	.763	.801	.842**	.884**	1.06	1.09	1.15*	1.22***
	Relative (%)	.307	.328	.374**	.408**	.347	.370	.413**	.517***
	Thyroid								
	Absolute (g)	.017	.019	.023**	.035**	.022	.033	.028**	.039***
	Relative (%)	.007	.008	.010**	.016**	.007	.012	.010***	.017***
	Spleen								
	Absolute (g)	.458	.499	.442	.423	.635	.901	1.282	1.084*
	Relative (%)	.185	.204	.195	.196	.205	.299	.482	.48***

*p<0.05; ** p<0.001; *** p<0.001; [+] Only 2 males at 24 month so statistic analysis was not possible.

Gross pathology: At month 12, treatment related changes were found in the liver (brownish-black) and kidneys (granular surface, brownish-black) of 6000 ppm in both sexes. At month 24, granular kidneys were observed in 1200 ppm males, and brownish-black kidneys, enlarged thyroid, decreased pituitary masses and alopecia were observed in 6000 ppm females. Rats that died during the course of the study also had treatment related swelling thyroids (both sexes) and granular kidneys (male).

Histopathology: Dose-related changes were found in the thyroid, liver, kidneys and adrenals. Thyroid follicular cell hyperplasia and hypertrophy were observed in both sexes at 1200 and/or 6000 ppm, as well as increases of focal follicular hyperplasia in 6000 ppm in both sexes. C-cell adenoma and C-cell adenocarcinoma were noted but not considered to be treatment related as high frequency was also seen in the control group. Adenoma was seen in both sexes at 1200 ppm (4/60 in males, 1/60 in females) and 6000 ppm (12/60 in males, 2/60 in females) but only one in male controls (1/60). Adenocarcinoma was seen in 6000 ppm males (3/60) but not in females. In rats that died on study, adenoma was seen in ≥1200 ppm

males and females. Adenocarcinoma was seen in ≥1200 ppm males but not in females (Table 20).

Epididymis mesothelioma was seen in males at 1200 ppm (1/23) and 6000 ppm (2/53) in rats that died or were sacrificed during the course of the study.

Table 20: Significant histopathological findings in thyroid (n=60)

	Incidence of thyroid follicular cell (FC) changes				
Males	**0 ppm**	**75 ppm**	**200 ppm**	**1200 ppm**	**6000 ppm**
Diffuse FC hyperplasia/hypertrophy	0/60 (0)	0/58 (0)	0/60 (0)	23/60 (38) **	58/60 (97) ***
Focal FC hyperplasia	3/60 (5)	2/58 (3)	2/60 (3)	3/60 (5)	15/60 (25) **
FC adenoma	1/60 (2)	0/58 (0)	0/60 (0)	4/60 (7)	12/60 (20) **
FC adenocarcinoma	0/60 (0)	0/60 (0)	0/60 (0)	0/60 (0)	3/60 (5)
Females	**0 ppm**	**75 ppm**	**200 ppm**	**1200 ppm**	**6000 ppm**
Diffuse FC hyperplasia/hypertrophy	1/60 (2)	1/59 (2)	0/60 (0)	28/60 (47) ***	59/60 (98) ***
Focal FC hyperplasia	3/60 (5)	1/59 (2)	0/60 (0)	4/60 (7)	8/60 (13) *
FC adenoma	0/60 (0)	0/59 (0)	0/60 (0)	1/60 (2)	2/60 (3)
FC adenocarcinoma	0/60 (0)	0/59 (0)	0/60 (0)	0/60 (0)	0/60 (0)

Note: Values in brackets are percentage

In the liver, centrilobular hepatocellular hypertrophy and lipofuscin were observed in both sexes of ≥1200 ppm at month 12 and 24. These changes were also observed in majority of animals treated at 6000 ppm of both sexes who died on study (Table 19).

In kidney, the severity of nephropathy was increased in 6000 ppm (both sexes) at month 12 and ≥1200 ppm (both sexes) at month 24. The changes mainly were numerous protein casts in the corticomedullary junction with interstitial fibrosis, regenerative tubules, thickened tubular basement membrane and glomerular lesions.

In addition, most 6000 ppm males died or were killed in extremis had severe nephropathy associated with hyperplasia of the parathyroid, demineralization of the bone and metastatic calcification in various organs.

Degeneration and atrophy of testes were seen in all groups at similar incidences and severity. This background incidence of testicular toxicity has the potential to mask treatment related effects on spermatogenesis observed in an earlier chronic dietary study in Sprague Dawley rats (Hashimoto & Tsubura 1972).

Conclusion: The NOEL was 200 ppm (9 mg/kg bw/d) based on reduced bodyweight gains, anaemia, increased urine protein, liver, kidney, and thyroid organ weights, and histopathological changes in thyroid, liver and kidney.

Auletta CS (1992). A chronic (1-year) oral toxicity study in the dog via capsule administration with thiophanate-methyl-subchronic. Laboratory: not specified. Report No: Doc. 537-002, RD-9207. Study duration: 6 September 1990 to 11 September 1991. Report date: 1992. Guidelines: OECD 452. GLP: Yes.

Materials and method: Thiophanate-methyl (purity 96.55%) was daily orally administered via gelatine capsules to Beagle dogs (4/sex/group, 6 month old, bw: 6.5-10.9 kg) at dose levels of 0, 8, 40 or 200 mg/kg bw/d for 12 months. Signs of clinical toxicology, mortality, were observed daily, food consumption and bodyweight observed weekly. Ophthalmoscopic examinations were carried out on all animals prior to study and at the end of the study. Blood was taken from all animals for haematology and clinical chemistry at 0, 1.5 and 3 month and at the end of the study. Urinalysis was performed at 0, 3 and 6 months and at the end of the study. All animals were sacrificed for gross and microscopic examinations at the end of the study.

Observations: There were no deaths or treatment-related clinical signs. Tremors were seen in all dogs at the high dose 2-4 h after treatment on one or more occasions during the initial three weeks of the study but not subsequently. Reduced bodyweight gains were observed in both sexes at 40 and 200 mg/kg bw/d (19% and 55% lower respectively for males; 15% and 64% lower respectively for females). Food consumption was only slightly reduced in males at 200 mg/kg bw/d (less than 10%). No abnormalities were found in ophthalmoscopic examination and urinalysis.

Haematology and clinical chemistry: Haematological effects consisted of slight decreases in total erythrocyte counts and haemoglobin and hematocrit values in males at 200 mg/kg bw/d. Changes in a number of clinical chemistry parameters were slight in magnitude and/or were within the historical control range for dogs at similar ages and/or without an apparent dose-response pattern. Thus, most of these haematological and clinical chemistry changes were not considered toxicologically significant except for cholesterol and thyroxine levels. Thyroid function tests revealed decreased thyroxine (T4) levels in at 40 and 200 mg/kg bw. There were no clear effects on triiodothyronine (T3) or thyroid-stimulating hormone (TSH).

Pathology: Increased relative liver weights were observed in both sexes at 200 mg/kg bw/d while increased relative thyroid weights were seen in both sexes at 40 and 200 mg/kg bw/d. Microscopic alterations attributed to treatment were limited to minimal to moderate hypertrophy and slight hyperplasia of the follicular epithelium of the thyroid at 40 and 200 mg/kg bw/d (Table 21).

Table 21: Significant findings (doses in mg/kg bw/d)

Parameter	Male				Female			
	0	8	40	200	0	8	40	200
Haematology (n=4)								
RBC (mil/µl)								
Pre-treatment	6.1	6.4	6.1	5.9	6.5	6.53	6.8	6.5
Week 52	7.6	7.7	7.3	6.6**	7.6	7.2	7.3	7.2
Hb(g/dl)								
Pre-treatment	13.5	14.2	13.2	13.2	14.4	14.4	14.8	1744
Week 52	17.4	17.5	16	15**	17	17	16	16.5
Ht (%)								
Pre-treatment	42	45	41	41	44	44	46	45
Week 52	52	53	48	45**	52	49	52	51

Clinical chemistry (n=4)								
T4 (ng/mL)								
Pre-treatment	1.35	1.36	1.37	1.35	1.3	1.35	1.26	1.27
Week 13	1.38	1.51	1.21*	1.08**	1.8	1.55	1.34**	1.35**
Organ weights (n=4)								
Bodyweight (kg)	13	-	12.1	10.4	10.7	-	9.9*	8.5**
Thyroid								
Absolute (g)	0.93	-	1.24	1.31	0.77	-	1.0*	0.998
Relative (x10000)	0.73		1.04**	1.27**	0.68		1.04**	1.2**
Liver								
Absolute (g)	308	-	-	364	272	-	-	288
Relative (x1000)	2.4			3.5*	2.6			3.5*
Histology (n=4)								
Thyroid								
Follicular cell hypertrophy	0	0	3	4	0	0	2	4
Follicular cell hyperplasia	0	0	1	4	0	0	0	3

*p <0.05, ** p <0.01; - data not included

Conclusion: The NOEL was 8 mg/kg bw/d for dogs in both sexes for the 1-year study, based on decreased levels of thyroxine (T4), increased relative thyroid weights and incidences of hypertrophy and hyperplasia in the follicular epithelium of the thyroid at 40 and 200 mg/kg bw/d.

6. REPRODUCTION STUDIES

Müller W (1993) Thiophanate-methyl: Two-generation oral (dietary administration) reproduction toxicity study in the rat. Hazleton Deutschland, Munster, Germany. Unpublished Study No. 683-004; Report date: 27 August 1993.

Test Chemical:	Thiophanate-methyl (95.9% purity)
Test Species:	25 ♂ and 25 ♀ Sprague Dawley rats/group; mean bw: 160-260 g; source: Charles River Wiga, Sulzfeld, Germany
Duration of Study:	January 1992- May 1993
QA & GLP:	Yes
Guidelines:	EPA Guideline 83-4

Materials and methods: Groups of rats (25/sex/dose) continuously received thiophanate-methyl in the diet at 0, 200, 630 or 2000 ppm throughout 2 generations. F0 rats were dosed for 14 weeks before mating and then continuously dosed until scheduled sacrifice at the end of the lactation period (21 days). The F0 females were allowed to litter and to rear their offspring (F1) to weaning. F1 pups were selected and dosed for 14 weeks from weaning to mating and continuously dosed until scheduled sacrifice at the end of the lactation period. The F1 females were allowed to litter and to rear their offspring (F2a) to weaning. After weaning of the F2a pups, a second mating of the F1 generation was employed to produce F2b. The animals were observed daily for mortality and clinical signs, while food consumption and bodyweight were recorded weekly (during pre-mating, gestation and lactation periods). Reproductive performance indices (mating, fertility and pregnancy) were determined for F0 and F1 parental animals. F0 and F1 parental animals were necropsied five and two weeks after weaning,

respectively and organs were weighed (brain, ovaries, testes, kidneys, liver, epididymides, prostate, pituitary, seminal vesicles, thyroid and uterus) and tissues examined microscopically (as the above plus vagina and all gross lesions). The F1 and F2 pups were examined daily for mortality and clinical signs and were weighed on days 1, 4, 7, 14 and 21 of lactation. The number of pups delivered and the sex ratio were determined and the viability index was recorded on lactation days 1, 4, 7, 14 and 21. Following weaning, 5 pups/sex/litter were randomly selected for necropsy and histopathological examination, while the remaining pups were sacrificed and organs and tissues examined macroscopically.

Daily intake of Thiophanate-methyl (mg/kg bw/d) (parental animals) during the pre-mating period

Dose (ppm)	200	630	2000
F0 males	11	36	115
F0 females	14	45	140
F1 male	11	35	114
F1 females	13	41	128

Parental animals: Four deaths of the F0 females were recorded. One control female was found dead on day 136 without showing any clinical signs on the days prior to its death. Two females at 200 ppm were found dead on day 138 and 155 respectively. The former showed poor physical condition, lacrimation and necropsy findings revealed the thoracic and abdominal cavity were filled with red fluid contents. The other female in this group did not show any clinical signs and necropsy findings were not detected. At 630 ppm, one female died during delivery on day 122. Necropsy revealed findings in the kidneys, stomach, lungs and thoracic cavity. No treatment-related clinical signs were detected in other animals. Three deaths (2 males and one female at 630 ppm) occurred in the F1 animals. Necropsy of these animals did not reveal any significant findings.

Bodyweight gains were reduced in F0 males during prior to, during and after mating and in F1 females during gestation at 2000 ppm (10% and 12% lower respectively). Food consumption was decreased at 2000 ppm in F1 females during gestation in first and second matings (12% lower). Decreased levels in thyroid hormones (T3 and/or T4) in both sexes were observed at 2000 ppm in F0 but not in F1 animals (Table 22). The lower levels of thyroid hormones were compensated by increased levels of stimulating thyroid hormone (TSH). Although T3 and T4 levels were not affected in the F1 animals, the levels were increased in both sexes at 2000 ppm. Relative liver and thyroid weights were increased in males of both generations and in females of the first generation at 2000 ppm. Correspondingly, increased incidences of hepatocyte hypertrophy and follicular cell hypertrophy and hyperplasia were seen in males of both generations and females of the first generation at 2000 ppm (Table 23). Mating performance, fertility and pregnancy indices were not affected by treatment.

Offspring: The only significant finding was lower bodyweight in F2b pups at 2000 ppm (12% lower).

Table 22: Significant thyroid hormone findings (n=10; dose in ppm)

		Male				Female			
		0	**200**	**630**	**2000**	**0**	**200**	**630**	**2000**
T3 (ng/mL)									
F0 adult	Week 1	0.64	0.63	0.59	0.51	0.82	0.81	0.81	0.77
	Week 8	0.50	0.56	0.61	0.57	0.7	0.65	0.63	0.63
	At necropsy	0.7	0.79	0.80	0.74	0.84	0.77	0.76	0.71*
T4 (µg/100 mL)									
	Week 1	4.8	4.4	4.3	3.5*	4.5	4.2	3.8	3.2*
	Week 8	5.2	5.0	5.6	4.5*	3.9	3.4	3.6	3.3
	At necropsy	4.2	3.9	4.7	4.3	2.7	2.7	3.1	2.8
	TSH (ng/mL)								
	Week 1	2.8	3.7	3.4	4.9*	1.7	1.8	1.6	1.8
	Week 8	4.2	5.7	4.6	7.9*	1.4	1.8	1.8	3.3*
	At necropsy	2.8	3.1	3.3	5.1	3.1	2.3	3.1	3.5
TSH (ng/mL)									
F1 adult	Week 8	3.4	4.1	5.3	6.9*	1.5	1.8	2.2	3.6*
	At necropsy	4.5	3.6	3.8	4.2	3.4	3.3	2.8	4.2

*p<0.05

Table 23: Significant organ weight and histological findings (n=25; dose in ppm)

		Male				Female			
		0	200	630	2000	0	200	630	2000
Organ weight (g)									
F0 adult	Bodyweight (g)	643	630	617	622	326	315	329	322
	Liver								
	Absolute (g)	24.4	25	24.7	28.8*	12.1	12.1	12.0	13.6*
	Relative (%)	3.8	3.9	4.0	4.6*	3.7	3.8	3.6	4.2*
	Thyroid								
	Absolute (g)	0.03	0.03	0.03	0.04*	0.023*	0.022*	0.023*	0.03*
	Relative (x1000)	0.046	0.047	0.048	0.06*	0.07	0.07	0.07	0.09*
F1 adult	Bodyweight (g)	640	672	612	603	357	365	356	356
	Liver								
	Absolute (g)	20.6	25.3	23	26	13.4	13.6	13.4	14.2
	Relative (%)	3.3	3.7	3.7.6	4.9*	3.7	3.7	3.7	3.9
	Thyroid								
	Absolute (g)	0.028	0.03	0.03	0.036*	0.023	0.026	0.026	0.028
	Relative (x1000)	0.044	0.044	0.049	0.06*	0.064	0.07	0.07	0.078
Histology									
F0 adult	Hepatocyte hypertrophy	0	0	0	22	0	0	0	18
	Follicular cell hypertrophy	0	0	0	22	0	0	0	6
	Follicular cell hyperplasia	1	0	0	21	0	0	0	5
F1 adult	Hepatocyte hypertrophy	0	0	0	6	0	0	0	0
	Follicular cell hypertrophy	0	0	0	20	0	0	0	0
	Follicular cell hyperplasia	1	0	0	24	0	0	0	0

*p<0.05

The NOEL was 630 ppm (approximately 35 mg/kg bw/d) for parents and offspring, based on decreased bodyweight gain and increased liver and thyroid weights (maternal) and decreased pup bodyweight at 2000 ppm.

Palmer AK (1972) Effect of thiophanate-methyl on reproductive function of multiple generations in rat. Huntington Research Centre, Huntington, England. Unpublished Study No. RD-73063; Report date: 30 May 1972.

Test Chemical:	Thiophanate-methyl (95.9% purity)
Test Species:	10 ♂ and 20 ♀ CD rats/group; mean bw: 70-80 g; source: Charles River Breeding, Wilmington. Massachusetts, USA
Duration of Study:	Not stated
QA & GLP:	Not stated
Guidelines:	Not stated

Materials and methods: Groups of rats continuously received thiophanate-methyl in the diet at 0, 40, 160 or 640 ppm. Treatment continued throughout the study for three generations and test animals (males and females) of each generation, F0, F1B and F2B were maintained on their respective diet for 60 days prior to mating. During the mating period (20 days0, animals were housed on the basis of one male to two females. Subsequently, the males were returned to their original cages while the females were transferred to individual cages for the birth and rearing of litters. Ten days following the weaning of the first litters, the animals were re-mated and a second litter produced. From the second litters of the initial (F0) and second (F1B) generations, 10 males and 20 females were selected from each group at weaning to form the basis of the second and third (F2B) generations, respectively. The animals were selected from as many litters and as possible. Brother and sister matings were avoided for the second and third generations. The animals were observed daily for mortality and clinical signs, while food consumption and bodyweight were recorded weekly (during pre-mating, gestation and lactation periods). Reproductive performance indices (mating, fertility and pregnancy) were determined for all generations. Parental animals were necropsied five and two weeks after weaning, respectively and organs were weighed (brain, bone, bone marrow, ovaries, testes, kidneys, liver, epididymides, prostate, pituitary, seminal vesicles, thyroid and uterus) and tissues examined microscopically (as the above plus vagina and all gross lesions). Pups were examined daily for mortality and clinical signs and were weighed on days 1, 4, 12 and 21 of lactation. The number of pups delivered and the sex ratio were determined and the viability index was recorded on lactation days 1, 4, 7, 14 and 21. Following weaning, 5 pups/sex/litter were randomly selected for necropsy and histopathological examination, while the remaining pups were sacrificed and organs and tissues examined macroscopically.

Results: In parental animals, the incidence of mortality, bodyweight change, food consumption, mating performance, pregnancy rate, duration of gestation and findings at terminal necropsy were not affected by treatment. There were no other treatment-related effects on the F1, F1B and F2B pups (number of pups delivered, viability index, clinical signs and histopathological findings).

The NOEL was 640 ppm.

7. DEVELOPMENTAL STUDIES

Rodwel D (1981a) Pilot teratogenicity study in rats. International Research and Development Corporation, Mattawan, Michigan, USA. Study No.: 449-005 (unpublished); Report date: 19 January 1981.

Test chemical:	Thiophanate-methyl (purity not stated)
Test species:	Female rats Crj:CD (SD), 12 w.o, (bodyweight not stated) from Charles River Laboratories, Inc. Portage, Michigan, USA.
Duration of study:	12 May-04 June 1980
GLP and QA :	Yes
Guidelines:	Not stated

Materials and methods: Pregnant rats (5/dose) were administered thiophanate-methyl (in 5% Arabic Gum vehicle) orally by gavage at 0, 250, 500, 1000, 3000 or 5000 mg/kg bw/d from days 6 to 19 of gestation. The animals were observed 1-2 times daily for mortality and clinical signs while bodyweights were recorded on days 0, 6, 9, 12, 16 and 20. On day 20, the rats were necropsied and the organs of the thoracic, abdominal cavities and pelvic viscera were grossly examined. The uterus including contents was weighed and implants (live, dead and resorption) in the uterus and numbers of corpora lutea in each ovary were recorded. The abdominal and thoracic cavities and organs of the dams were examined and discarded.

Results: No deaths or clinical signs observed during the study. Bodyweight gains were reduced in a dose-related manner at 1000, 3000 and 5000 mg/kg bw/d (15%, 13% and 17% lower, respectively). There were no dose-related trends in the mean number of corpora lutea, total implantations, viable foetuses or post-implantation loss in any treatment groups. Based on the results of this study, dosage levels of 0, 100, 300 and 1000 mg/kg bw/d were selected for a teratology study.

Rodwel D (1981b) Teratogenicity study of Thiophanate-methyl in rats. International Research and Development Corporation, Mattawan, Michigan, USA. Study No.: 449-006 (unpublished); Report date: 29 January 1981.

Test chemical:	Thiophanate-methyl (purity not stated)
Test species:	Female rats Crj:CD (SD), 12 w.o, (bodyweight not stated) from Charles River Laboratories, Inc. Portage, Michigan, USA.
Duration of study:	28 July-04 September 1980
GLP and QA :	Yes
Guidelines:	Not stated

Material and methods : Pregnant rats (5/dose) were administered thiophanate-methyl (in 5% Arabic Gum vehicle) orally by gavage at 0, 100, 300 or 1000 mg/kg bw/d from days 6 to 19 of gestation. The animals were observed 1-2 times daily for mortality and clinical signs, while food consumption and bodyweight were recorded on days 0, 6, 9, 12, 16 and 20. On day 20, the rats were necropsied and the organs of the thoracic, abdominal cavities and pelvic viscera were grossly examined. The uterus including contents was weighed and implantations (live, dead and resorption) in the uterus and numbers of corpora lutea in each ovary were recorded. Live foetuses were numbered, weighed, sexed and examined for external alterations

(malformation, anomaly and variation). Foetuses were assigned to either visceral or skeletal evaluation at an approximate 1:1 ratio within each litter.

Results: No deaths or clinical signs observed during the study. Bodyweight gains were reduced at 1000 mg/kg bw/d (9% lower). There were no dose-related trends in the mean number of corpora lutea, total implantations, viable foetuses or post-implantation loss in any treatment groups. Examination of the ovaries and uterine contents at caesarean section on day 20 revealed no treatment-related adverse effects. Incidences of foetal malformation and variation were similar among groups.

The NOEL was 300 mg/kg bw/d for maternal toxicity based on reduced bodyweight gains at 1000 mg/kg bw/d. The NOEL for developmental toxicity was 1000 mg/kg bw/d, the highest dose tested. There was no treatment-related increase in foetal malformations.

Tesh JM (1986) Thiophanate-methyl: Teratogenicity study in the rabbit. Life Science Research, Suffolk, England. Study No.: 86/NISO10/111 (unpublished) Report date: 20 May 1986.

Test chemical:	Thiophanate-methyl (purity 96.2%)
Test species:	Female NZW rabbits, 4.5 m.o, (bodyweight: 3.5-4.7 kg from Ranch Rabbits, Crawley Down, Sussex, England
Duration of study:	28 August-09 December 1985
GLP and QA:	Yes
Guidelines:	Not stated

Material and methods: Pregnant rabbits (15/dose) were administered thiophanate-methyl (in 1% (w/v) aqueous methylcellulose vehicle) orally by gavage at 0, 2, 6 or 20 mg/kg bw/d from days 6 to 28 of gestation. The animals were observed 1-2 times daily for mortality and clinical signs. Bodyweight was recorded on gestation days 0, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 28, while food consumption was recorded regularly. On day 29, the rats were necropsied and the organs of the thoracic, abdominal cavities and pelvic viscera were grossly examined. The uterus including contents was weighed and implants (live, dead and resorption) in the uterus and numbers of corpora lutea in each ovary were recorded. Live or dead foetuses were numbered, weighed, sexed and examined for external alterations (malformation, anomaly and variation). Foetuses were then sacrificed and subject to visceral and skeletal evaluation.

Maternal toxicity: During the gestation period**,** two animals in the control group and one in each of the treated groups were euthanised *in extremis*. In all cases, necropsy revealed no findings. No treatment-related clinical signs were observed. Bodyweights at 6 and 20 mg/kg bw/d were reduced during the first 2-8 days of treatment but recovered thereafter (5% and 9% lower). Food consumption during this period at 20 mg/kg bw/d was lower than the control group (13% and 30% lower). Reduction in bodyweight at 6 mg/kg bw/d was not considered to be treatment-related since the effect was marginal (less than 10%) and was not observed in another developmental rabbit study at 5 and 10 mg/kg bw/d (York 1997b). Examination of the ovaries and uterine contents at caesarean section on day 29 revealed no treatment-related adverse effects.

Developmental toxicity: An increased incidence of foetuses with 13 pairs of ribs and/or 27 presacral vertebrae was observed at 6 and 20 mg/kg bw/d (Table 24). The incidences were within, or on borderline with, the historical control incidences for New Zealand White rabbits

(from 86 studies) and were not observed at 20 mg/kg bw/d in another developmental study in rabbits (York 1997b) and thus were not considered to be treatment-related. Treatment did not affect foetal survival (live foetuses/dam), sex ratio, foetal weight or malformations.

Table 24: Incidences of foetal skeletal variations (incidence/litter)

Dose (mg/kg bw/d)	0	2	6	20	Historical control (incidence)
Number of foetuses/litters examined	**88/12**	**78/10**	**95/12**	**51/9**	
Number of ribs (13/13)	41/10	46/10	62/11	61/8	12-61
Number of presacral vertebrae 27	16/7	18/8	38/9	43/7	7-44

The NOEL was 6 mg/kg bw/d for maternal toxicity based on reduced bodyweight in the dams at 20 mg/kg bw/d. The NOEL for developmental toxicity was 20 mg/kg bw/d. There was no treatment-related increase in foetal malformations.

York RG (1997a) Oral dose-range developmental toxicity study of thiophanate-methyl in rabbit. Angus Research Laboratories, Horsham, Pennsylvania, USA. Study No.: 914-002P (unpublished) Report date: 11 August 1996.

Test chemical:	Thiophanate-methyl (purity 97.28%)
Test species:	Female NZW rabbits, 6 m.o, (bodyweight: 3.2-4.5 kg from Covance Research Products, Denver, Pennsylvania, USA
Duration of study:	16 February-14 March 1997
GLP and QA:	Yes
Guidelines:	EPA 83-3

Material and methods: Pregnant rabbits (6/dose) were administered thiophanate-methyl (in 1% (w/v) aqueous methyl cellulose vehicle) orally by gavage or in the diet at 0, 5, 10, 20, 40 or 80 mg/kg bw/d from days 6 to 28 of gestation (for dietary exposure the doses were based on concentration of 0, 97, 194, 389, 788 or 1556 ppm). The animals were observed 1-2 times daily for mortality and clinical signs. Bodyweight was recorded on gestation days 0, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 29 while food consumption was recorded regularly. On day 29, the rats were necropsied and the organs of the thoracic, abdominal cavities and pelvic viscera were grossly examined. The uterus including contents was weighed and implants (live, dead and resorption) in the uterus and numbers of corpora lutea in each ovary were recorded. Live or dead foetuses were numbered, weighed, sexed and examined for external alterations (malformation, anomaly and variation). Only foetuses from three gavage groups (0, 5 and 80 mg/kg bw/d) were subject to visceral and skeletal evaluation. Since this is a dose-range study, no statistical analysis of the data was performed.

Maternal toxicity; No deaths or clinical signs were observed. In the dietary groups, seven does aborted (one, three and three does) in at 5, 40 and 80 mg/kg bw/d dietary groups, respectively. In the gavage groups, two does aborted at 80 mg/kg bw/d. Bodyweight gains were reduced in all gavage treated groups in a dose-related manner (ranged from 18-78% lower) and at 20 mg/kg bw/d and higher in dietary groups (ranged from 18-98% lower). Food consumption was reduced in all gavage treated groups in a dose-related manner (ranged from 18-52% lower) and at 20 mg/kg bw/d and higher in dietary groups (ranged from 15-70% lower). The highest dose in both dietary and gavage groups had increased numbers of early resorptions and decreased live litter sizes (Table 25).

Table 25: Reproductive parameters (%)

Dose (mg/kg bw/d)	0	5	10	20	40	80
Dietary group						
Live foetuses	7.7	8	9.8	7	9.5	0
Early resorption	0.3	0	0.2	0.2	0.5	5
Gavage group						
Live foetuses	8.7	7.2	8.2	8.6	8.4	5.2
Early resorption	00	0.2	0.4	0.2	0	3.2

Developmental toxicity: The number of thoracic vertebrae was increased and lumbar vertebrae were decreased at 80 mg/kg bw/d in the gavage group. The number of rib pairs was also increased at this dose. Treatment did not affect foetal survival (live foetuses/dam), sex ratio or foetal weight or malformations.

Since developmental toxicity was only determined for three groups (0, 5 and 80 mg/kg bw/d), no NOEL was established.

York RG (1997b) Oral developmental toxicity study of thiophanate-methyl in rabbit. Angus Research Laboratories, Horsham, Pennsylvania, USA. Study No.: 914-002 (unpublished) Report date: 11 August 1996.

Test chemical:	Thiophanate-methyl (purity 97.28%)
Test species:	Female NZW rabbits, 6 m.o, (bodyweight: 3.2-4.5 kg from Covance Research Products, Denver, Pennsylvania, USA
Duration of study:	16 February-14 March 1997
GLP and QA:	Yes
Guidelines:	EPA 83-3

Material and methods: Pregnant rabbits (20/dose) were administered thiophanate-methyl (in 1% (w/v) aqueous methyl cellulose vehicle) orally by gavage at 0, 5, 10, 20 or 40 mg/kg bw/d from days 6 to 28 of gestation. The animals were observed 1-2 times daily for mortality and clinical signs. Bodyweight was recorded on gestation days 0, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 29 while food consumption was recorded regularly. On day 29, the rats were necropsied and the organs of the thoracic, abdominal cavities and pelvic viscera were grossly examined. The uterus including contents was weighed and implants (live, dead and resorption) in the uterus and numbers of corpora lutea in each ovary were recorded. Live or dead foetuses were numbered, weighed, sexed and examined for external alterations (malformation, anomaly and variation). Foetuses were then sacrificed and subjected to visceral and skeletal evaluation.

Maternal toxicity: No deaths, abortions or clinical signs were observed. Bodyweight gains were reduced at 20 and 40 mg/kg bw/d throughout the treatment period (8% and 69% lower, respectively). Food consumption during this period at 20 and 40 mg/kg bw/d was lower than the control group (12% and 54% lower, respectively). Examination of the ovaries and uterine contents at caesarean section on day 29 revealed no treatment-related adverse effects.

Table 26: Incidences of foetal skeletal variations

Dose (mg/kg bw/d)	0	5	10	20	40
Number of foetuses/litters examined	**168/19**	**141/17**	**164/18**	**115/16**	**160/19**
Vertebrae					
Thoracic	12.5	12.5	12.5	12.7	12.9**
Lumbar	6.5	6.5	6.5	6.4	6.1**
Ribs (pair)	12.4	12.4	12.4	12.5	12.8**

**p<0.01

Developmental toxicity: An increase in the average number of thoracic ribs (supernumerary ribs), with associated increases in the averages for thoracic vertebrae, was observed with an associated increase in the average number of thoracic vertebrae and decreased numbers of lumbar vertebrae at 40 mg/kg bw/d Table 26). The increased incidence of supernumerary ribs is likely to be linked to reduced maternal bodyweight gain. Treatment did not affect foetal survival (live foetuses/dam), sex ratio or foetal weight or malformations.

The NOEL was 10 mg/kg bw/d for maternal toxicity based on reduced bodyweight gains in the dams at higher doses. The NOEL for developmental toxicity was 20 mg/kg bw/d based on increased incidence of skeletal variations at 40 mg/kg bw/d. There was no treatment-related increase in foetal malformations.

8. GENOTOXICITY STUDIES

Table 27 summarises submitted and published findings of *in vitro* and *in vivo* genotoxicity studies for thiophanate-methyl. The *in vivo* studies are described in detail.

Table 27: Summary of Genotoxicity Studies

Assay/endpoints	Species, Strains	Concentration, Purity	Metabolic Activation	Result	Reference
In vitro					
Reverse mutation in bacteria	*S. typhimurium TA98, TA100, TA1535, TA1538, WP2uvrA*	625–5000 μg/plate, 96.5% purity	+,-	-	Kanaguchi *et al* (1990)
Gene mutation	Chinese hamster V79 cells	6.25-100 μg/mL, 96.5%	+,-	-	Munziata *et al* (1984)
Chromosomal aberrations	Chinese hamster ovary cells	70-1000 μg/mL, 99.5%	+,-	-	Murli *et al* (1991)
Unscheduled DNA synthesis	Rat hepatocytes	50-100 μg/mL, 99.5%	+,-	-	Myhr *et al* (1981)
Aneuploidy induction	Human lymphocytes	8-700 μg/mL, 99.7%	+,-	+ in the absence of metabolic activation	Marshall *et al* (1997a)
Aneuploidy induction	Human lymphocytes	0.05-39 μg/mL, 99.7%	+,-	+ ≥ 0.6 μg/mL (NOEL 0.4 μg/mL)	Marshall *et al* (1997b)
In vivo					
Micronucleus formation/Bone marrow micronucleus test	Mouse bone marrow	Single gavage dose of 1 g/kg bw thiophanate-methyl or 0.5 g/kg bw carbendazim	Not applicable	Equivocal	Barale *et al* (1993)
Micronucleus formation/Bone marrow micronucleus test	Mouse bone marrow	Single gavage dose of 0.5, 1 or 2 g/kg bw or 1 g/kg bw carbendazim	Not applicable	Equivocal	Proudlock *et al* (1999)
Micronucleus formation/Bone marrow micronucleus test	Rat bone marrow	Single gavage dose of 312, 625, 1250, 2500 or 5000 mg/kg bw/d for 5 days	Not applicable	No effects	Makita *et al* (1973)

Results (-, negative; +, positive) are expressed relative to the presence (+) or absence (-) of metabolic activation.

Barale R et al (1993) Cytogenetic effects of benzimidazoles in mouse bone marrow. Mutation Research 300:15-28.

Materials and Methods: In this report, the cytogenetic effects of three benzimidazoles, benomyl, thiophanate-methyl and carbendazim, were studied in mouse bone marrow cells by analysing three genetic endpoints: micronuclei, structural chromosome aberrations plus or minus gaps, and aneugenic effects (polyploidy). Male Swiss Albino mice (8 weeks old, 25 g) were administered a single gavage dose of either benomyl, thiophanate-methyl or carbendazim at 1, 1 or 0.5 g/kg bw respectively (4 mice for each chemical). Bone marrow erythrocytes were fixed and analysed.

Results: Benomyl, carbendazim and to a much lesser extent thiophanate-methyl caused an induction of micronuclei with a maximum at 38 hours after treatment (Table 28). A significant increase in chromatid gaps was observed for benomyl and carbendazim, but not thiophanate-methyl, between 6 and 18 hours after treatment. Chromatid gaps after carbendazim treatment were followed by definite chromosome damages such as breaks. By contrast, gaps induced by benomyl appeared not to involved breaks and then into micronuclei. For carbendazim, a second increase in chromatid gaps was seen at 30 hours after treatment. Carbendazim, and to a lesser extent, benomyl increased the number of polyploid cells around 30 hours after treatment. It was concluded that thiophanate-methyl was the least effective in relation to micronucleus induction as well as aneugenic effects.

Table 28: Micronucleus induction and structural aberrations in mouse bone marrow cells

Time (h)	MN/PCE (%)*				Aberrant cells with gaps (%)#				Polyploid cells#			
	C	BE	TM	CA	C	BE	TM	CA	C	BE	TM	CA
	1.7				2.2				0			
6		2.6		2.6		**7.5**		4.2		0		0
18		3.1	2.8	2.8		**6.8**	2.8	**7.6**		1	0	1
24		3.1	3.1	3.7		3.8	2.8	2.1		1	1	4
30		3.7	3	3.8		4	2.9	**7.1**		**6**	0	3
38		**5.4**	3	**6.4**		2.8	2.5	3.7		0	1	**4**
48		2.9	3	3.5		1.8	3.2	3.9		0	0	**17****

C: control; BE: benomyl; TM: Thiophanate-methyl; CA: carbendazim

*3000-4000: polychromatic erythrocyte (PCE)

#600: Metaphases scored

**combined results at 42 and 48 time points

Proudlock RJ (1999) Thiophanate-methyl mouse micronucleus test. Huntingdon Life Sciences, Cambridgeshire, England. Study No.: RD-9957 (unpublished). Report date: 10 May 1999.

Test chemical:	Thiophanate-methyl (purity 97.28%); carbendazim (purity (98%)
Test species:	B6D2F1 mice, 5 w.o, (bodyweight: 18-22 g from Charles River Limited, Kent, England
GLP and QA:	Yes
Guidelines:	OECD 474

Materials and method: This study was designed to investigate the potential induction of micronuclei in mouse bone marrow cells (5 sex/dose) following a single oral dose of thiophanate-methyl at 0, 500, 1000 or 2000 mg/kg bw or carbendazim at 2000 mg/kg bw.

Bone marrow smears were analysed 24 hours after treatment. A total of 2000 immature erythrocytes were examined for each mouse.

Results: There were no deaths or clinical signs. Compared to the controls, thiophanate-methyl induced a small increase in the frequency of micro-nucleated immature erythrocytes 24 hours after treatment. However, the effects were not dose-related. For thiophanate-methyl, the frequency of micro-nucleated immature erythrocytes ranged from 1-2, 2-7, 2-5 and 3-8 in every 2000 erythrocytes analysed, with a mean of 1.3, 4.2, 3.8 and 6.3 (or 0.06%, 0.2%, 0.19% and 0.3%) for control, 500, 1000 and 2000 mg/kg bw, respectively. The range of micro-nucleated immature erythrocytes observed was within the historical control range for mice (0-7 in every 2000 immature erythrocytes) provided by the applicant with 93% of individual mouse (total 729 mice) has a frequency of micro-nucleated immature erythrocytes between 0-3 while 7% has a frequency between 4-7. Carbendazim at 2000 mg/kg bw on the other hand, induced a large increase in the frequency of micro-nucleated immature erythrocytes ranging from 11-42 in every 2000 erythrocytes examined with a mean of 24.5. It was concluded that thiophanate-methyl was equivocal in relation to its ability to induce micronuclei in mouse immature erythrocytes.

Makita T et al (1973) Mutagenic, cytogenic and teratogenic studies on thiophanate-methyl. Tox. Applied. Pharmacol. 24:206-215.

Materials and method: In this report, the cytogenetic effects of were studied in rat bone marrow and spermatogonial cells. Wistar rats received a single dose of intraperitoneal injection at 8-500 mg/kg bw. For each dose, metaphase chromosomes from the bone marrow cells and the spermatogonial cells (100 cells each) were analysed. Colchicine (1 mg/kg bw) was used as the positive control.

Results: Cytogenetic effects such as breakage, chromosome translocation, fragmentation or micronuclei were not observed at any dose. The positive control gave appropriate results.

Marshall R (1997a) Thiophanate-methyl technical: Induction of micronuclei in cultured human peripheral blood lymphocytes. Corning Hazelton, North Yorkshire, England. Report number: RD-9728. Report date: February 1997.

Materials and method: The objective of this study was to evaluate the clastogenic and aneugenic potential of thiophanate-methyl in cultured human peripheral lymphocytes. Cells were treated with thiophanate-methyl at 0, 47, 50 or 63 μg/mL for 20 hours in the absence, or at 0, 450, 500 or 550 μg/mL for 3 hours in the presence, of rat liver post-mitochondria fraction (S9) followed by a recovery period of 17 hours. The frequency of micro-nucleated lymphocytes was analysed (2000 cells/dose). Colchicine and cyclophosphamide were employed as positive control chemicals in the absence and presence of S9, respectively.

Results: Thiophanate-methyl induced an increase in the frequency of micro-nucleated lymphocytes in the absence of S9. However, the effects were not dose-related. The frequency of micro-nucleated cells was 12, 43, 33 and 49 in every 2000 lymphocytes analysed for control, 47, 50 or 63 μg/mL, respectively. The range of micro-nucleated lymphocytes observed was outside the historical control range (2-13 in every 12000 lymphocytes in the absence of S9). In the presence of S9, the frequency of micro-nucleated cells was 5, 2, 7 and 13 in every 2000 lymphocytes analysed for control, 450, 500 or 550 μg/mL, respectively

which was within the historical control range (0-14 in every 14000 cells in the presence of S9). Positive controls gave appropriate results.

Marshall R (1997b) Thiophanate-methyl technical: Study to determine the threshold of action for the induction of aneuploidy in cultured human peripheral blood lymphocytes. Corning Hazelton, North Yorkshire, England. Report number: RD-9729. Report date: February 1997.

Materials and method: The objective of this study was to generate dose response data for thiophanate-methyl to determine if it was possible to demonstrate a threshold of action for the induction of aneuploidy in cultured human peripheral blood lymphocytes.

This study was poorly designed and written. The results were poorly presented making interpretation difficult. This study therefore, was not relied on.

9. MECHANISTIC STUDIES

Nishibe, T. & Takaori, H. (1990) Summary of mechanistic investigation of the effect of thiophanate-methyl on thyroid and liver. Unpublished report from the Toxicology Institute, Environmental Toxicology Laboratory, Nippon Soda Co. Ltd, Kanagawa, Japan. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

Thiophanate-methyl (purity, 96.55%) was administered in the diets of six-week-old male Fischer 344 rats for two or eight days at a concentration of 6000 ppm. Propylthiouracil, an inhibitor of thyroid hormone synthesis, was administered at 1000 ppm for two or eight days and phenobarbital, an inducer of drug-metabolizing enzymes, was administered at 500 ppm for eight days, as positive controls. Thiophanate-methyl reduced triiodothyronine and thyroxine levels, increased thyroid-stimulating hormone activity (at day 8 only), increased thyroid weights (at day 8 only), and increased liver weights. Propylthiouracil caused similar but more marked changes in thyroid hormone levels and weights. Phenobarbital increased liver weights. All three chemicals increased serum cholesterol levels by day 8. Thiophanate-methyl and phenobarbital increased microsomal cytochrome P450, cytochrome b5, total protein, and UDP-glucuronosyltransferase activities.

PART II: OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

1. PRODUCTS AND THEIR USE PATTERNS

At the commencement of this review there were three registered thiophanate-methyl products in Australia (Table 29): two wettable powder formulations (one containing 150g/kg etridiazole and 250g/kg thiophanate-methyl and one containing 640g/kg mancozeb and 156g/kg thiophanate-methyl), and one granular formulation (containing 30g/kg etridiazole and 50g/kg thiophanate-methyl). All three products are registered for the control of soil-borne diseases of ornamental plants and are applied either directly to the soil (evenly mixed with the soil or as a soil drench) or as a spray (Table 30). They are not registered for use on food-producing plants and the labels do not indicate that these products are for home garden use. Public and occupational health and safety assessments for the three products were conducted by OCSEH in 2000 and 2001. Following this review of thiophanate-methyl, it was considered that the existing PPEs and re-entry statement recommended for the three products remain appropriate and thus will not be considered any further in this section.

Table 29: Thiophanate-methyl products registered in Australia at commencement of review.

APVMA Product Code	Product Name	Registrant	Carbendazim or thiophanate-methyl content
52741	Banrot 400 WP Broad Spectrum Fungicide for Ornamentals	Scotts Australia Pty Ltd	250 g/kg
53163	Banrot 80 G Broad Spectrum Fungicide for Ornamentals	Scotts Australia Pty Ltd	50 g/kg
53760	Zyban WP Broad Spectrum Fungicide for Ornamental Plants	Scotts Australia Pty Ltd	156 g/kg

Table 30: Use patterns for thiophanate-methyl products

Crop	Pest	Description	Maximum rate	Critical comments
Container grown seedlings, cuttings, transplants – pre-plant soil mix additive	Damping off rot and root and stem diseases caused by – *Pythium, phytophthora and thielaviopsis (chalara)*	50 g/kg (GR) 250g/kg (WP)	50 g/kg 60g/cubic metre	Mix evenly into the soil/potting mix before sowing or transplanting. An even mix is essential for effective disease control. Pre-mixing into a small sample of the soil/potting mix before adding may assist even application. One treatment will give control for 4-8 weeks over the establishment or germination period. If longer protection is required then retreat at 4-8 week intervals using broadcast or soil drench.
Container grown seedlings, bedding plants, shrubs and other woody plants, and indoor plants		250g/kg (WP)	2-4kg/100 sq m (post- plant broadcast treatment) 400-800g/100 sq metres in 1000L water (soil drench)	Measure the area covered by the pots/containers to be treated. Weigh out the amount of product required. Apply evenly over the measured area using a calibrated granule spreader. Irrigate within several days of application to incorporate the granules into the soil. Normal sprinkler irrigation will then continue to move the product into the soil. Or mix in with sufficient water to saturate the top 3-5 cms of soil. Apply evenly over measured area using a low pressure rose type applicator. Then irrigate with additional water equal to at least half the volume of fungicidal drench. Re-treat at 4-8 week intervals depending on the disease incidence. Lower rate when lower disease pressure is expected and vice versa.

Crop	Pest	Description	Maximum rate	Critical comments
In-ground bedding and other plants – post-plant broadcast treatment				Apply as for container grown plants. Harrow, rake or till into the soil to incorporate the granules into the upper 5-15cms of soil. Retreat at 4-8 week intervals. Lower rate when lower disease pressure is expected and vice versa.
In-ground bedding and other plants – pre-plant treatment				Apply as for container grown plants. Rotary hoe, harrow or till into the soil to incorporate the granules into the upper 5-15cms of soil. Retreat 4-8 week intervals. Lower rate when lower disease pressure is expected and vice versa.
Ornamental plants – shrubs, bedding plants, indoor plants eg.African violet, azalea, begonia, carnation, chrysanthemum, dahlia, ferns, geranium, gladioli, hollyhock, poppy, roses, snapdragon, zinnia	eg.Grey mould (*Botrytis cinerea*), petal blight (*Ovulinia azaleae),* powdery mildew (*Erysiphe cichoraceanum, Oidium* spp), rust (*Uromyces* spp, P*uccinia* spp.*),* fungal leaf spots/ blights (*Altenaria, mycosphaerella and septoria* spp.*)*, downy mildew (*Peronospora* spp),black spot (*Diplocarpon* spp)	156 g/kg (WP)	170g/100L water	Apply as a high volume spray to the point of run-off covering all foliage, stems and flowers. Commence spraying on the first appearance of disease or when conditions are suitable for disease infection. Apply at intervals of 7-14 days. Use shorter interval under severe disease pressure or when heavy rain has occurred since the last spray and the longer interval under lighter disease pressure.

2. ASSESSMENT OF EXPOSURE AND RISK -OCCUPATIONAL

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

Public and occupational health assessments for the three thiophanate-methyl products were conducted by OCSEH in 2000 and 2001. As a result, no further evaluation of occupational exposure and risk during mixing/loading and application was conducted by OCSEH.

3. SAFETY DIRECTIONS

At the time of this review, there were three registered thiophanate-methyl products in Australia: Banrot 400WP Broad Spectrum Fungicide for Ornamentals (containing 150 g/kg etridiazole and 250 g/kg thiophanate-methyl), Banrot 80G Broad Spectrum Fungicide for Ornamentals (containing 30 g/kg etridiazole and 50 g/kg thiophanate-methyl) and Zyban WSP Broad Spectrum Fungicide for Ornamental Plants (containing 640 g/kg mancozeb and 54 g/kg thiophanate-methyl). The three products are registered for the control of soil-borne diseases of ornamental plants and are applied either directly to the soil (evenly mixed with the soil or as a drench) or as a spray. The labels do not indicate that these products are for home garden use. Public and occupational health assessments for the three products were conducted by OCSEH in 2000 and 2001. The current safety directions for the three Australian products containing thiophanate-methyl are shown below.

Existing Safety Directions for thiophanate-methyl products

Thiophanate methyl HG LD 1.5 g/L or less	
210 211	Avoid contact with eyes and skin.
219 223	Avoid inhaling spray mist.
351	Wash hands after use.
Thiophanate methyl WP 700 g/kg or less	
210 211	Avoid contact with eyes and skin.
220 221 223	Do not inhale dust or spray mist.
351	Wash hands after use.

Etridiazole WP 161 g/kg or less with thiophanate-methyl 270 g/kg or less	
Codes	*Safety Directions*
161 162 164	Will irritate eyes and skin
210 211	Avoid contact with eyes and skin
279 280 281 282 290 292 294 297	When opening the container, preparing the spray and using the prepared spray, wear cotton overalls buttoned to the neck and wrist, elbow-length PVC gloves and goggles.
340 342	If product on skin, immediately wash area with soap and water.
340 343	If product in eyes, wash it out immediately water.
351	Wash hands after use
360 361 363 366	After each days use, wash gloves, goggles and contaminated clothing.
Etridiazole GR 34 g/kg or less with thiophanate-methyl 57 g/kg or less	
Codes	
161 162 164	Will irritate eyes and skin
210 211	Avoid contact with eyes and skin
279 280 283 290 292b 294 306	When opening the container and using the product, wear cotton overalls buttoned to the neck and wrist, and elbow-length PVC gloves and a disposable dust mask covering mouth and nose.
351	Wash hands after use
360 361 366	After each days use, wash gloves and contaminated clothing
Mancozeb WP 700 g/kg or less with thiophanate-methyl 200 g/kg or less when packed in sealed water soluble sachets (application by hand spray)	
Codes	*Safety Directions*
161 162	Will irritate eyes
210 162	Avoid contact with eyes
279 282 290 292 295 298	When using the prepared spray, wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length nitrile or PVC gloves and impervious footwear
340 343	If product in eyes, wash it out immediately water
351	Wash hands after use
360 361 366	After each day's use, wash gloves and contaminated clothing
Mancozeb WP 700 g/kg or less with thiophanate-methyl 200 g/kg or less when packed in sealed water soluble sachets (application by mechanical sprayer)	
Codes	*Safety Directions*
161 162	Will irritate eyes
210 162	Avoid contact with eyes
279 282 290 292b 295	When using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow-length nitrile or PVC gloves
340 343	If product in eyes, wash it out immediately water
351	Wash hands after use
360 361 366	After each day's use, wash gloves and contaminated clothing

Following this review of thiophanate-methyl, the existing safety directions including PPEs for the three registered products remain appropriate, except that PVC gloves are to be replaced by chemical resistant gloves, and the two entries for thiophanate methyl alone (HG LD 1.5 g/L or less and WP 700 g/kg or less) should be deleted as there are no registered products.

3. CONCLUSIONS AND RECOMMENDATIONS

1. The OCSEH recommends that the APVMA should be satisfied that persons involved in preparing and applying thiophanate-methyl-based products, according to label directions, will not suffer from adverse effects.

2. The following uses of thiophanate-methyl are supported without change to the current conditions of application:

- Application to ornamental plants by mechanical tractor and hand-held equipment.
- Application to soil.

3. A re-entry interval of 12 hours is recommended for persons performing management activities and persons should wear chemical resistant gloves and overalls if prior entry is required.

4. It was considered that the following existing Safety Directions in the FAISD Handbook remain appropriate, except that PVC gloves are to be replaced by chemical resistant gloves.

Amended Entries

Etridiazole WP 161 g/kg or less with thiophanate-methyl 270 g/kg or less	
Codes	*Safety Directions*
161 162 164	Will irritate eyes and skin
210 211	Avoid contact with eyes and skin
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately water
279 280 281 282 290 292 294c 297	When opening the container and preparing mix/drench and using the prepared mix/drench, wear cotton overalls buttoned to the neck and wrist and a washable hat and chemical resistant gloves and goggles
351	Wash hands after use
360 361 363 366	After each days use, wash goggles, gloves and contaminated clothing
Etridiazole GR 34 g/kg or less with thiophanate-methyl 54 g/kg or less	
Codes	
161 162 164	Will irritate eyes and skin
210 211	Avoid contact with eyes and skin
279 280 283 290 292b 294c 306	When opening the container and using the product, wear cotton overalls buttoned to the neck and wrist, and chemical resistant gloves and a disposable dust mask
351	Wash hands after use
360 361 366	After each days use, wash gloves and contaminated clothing
Mancozeb WP 700 g/kg or less with thiophanate-methyl 200 g/kg or less when packed in sealed water soluble sachets (application by hand spray)	
Codes	*Safety Directions*
161 162	Will irritate the eyes
210 162	Avoid contact with eyes
279 282 290 292b 294c 298	When using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and elbow length chemical resistant gloves and impervious footwear
340 343	If product in eyes, wash it out immediately water
351	Wash hands after use
360 361 366	After each day's use, wash gloves and contaminated clothing

Mancozeb WP 700 g/kg or less with thiophanate-methyl 200 g/kg or less when packed in sealed water soluble sachets (application by mechanical sprayer)	
Codes	*Safety Directions*
161 162	Will irritate the eyes
210 162	Avoid contact with eyes
279 282 290 292b 295	When using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow length chemical resistant gloves
340 343	If product in eyes, wash it out immediately water
351	Wash hands after use
360 361 366	After each day's use, wash gloves and contaminated clothing

Deleted entries

There are no products registered for these entries, therefore they should be deleted:

~~**Thiophanate methyl HG LD 1.5 g/L or less**~~	
210 211	Avoid contact with eyes and skin.
219 223	Avoid inhaling spray mist.
351	Wash hands after use.
~~**Thiophanate methyl WP 700 g/kg or less**~~	
210 211	Avoid contact with eyes and skin.
220 221 223	Do not inhale dust or spray mist.
351	Wash hands after use.

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APPENDIX I: 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 II: 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 III: Reproductive and Developmental Indices

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

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

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

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

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

* 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

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

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

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

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

* 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)

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

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

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

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

APPENDIX IV: 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