THIOPHANATE-METHYL (addendum)

First draft prepared by
Rudolf Pfeil[1] and David Ray[2]

[1] Federal Institute for Risk Assessment, Berlin, Germany; and
[2] Medical Research Council Applied Neuroscience Group, Biomedical Sciences, University of Nottingham, Queens Medical Centre, Nottingham, England

Explanation

Thiophanate-methyl is the International Organization of Standardization (ISO) approved common name for dimethyl 4,4'-(*o*-phenylene)bis(3-thioallophanate), a systemically active benzimidazole fungicide that inhibits the synthesis of β-tubulin. Thiophanate-methyl was previously evaluated by the Joint Meeting on Pesticide Residues (JMPR) in 1973, 1975, 1977, 1995 and 1998. In 1998, an acceptable daily intake (ADI) of 0–0.08 mg/kg bw was established based on the no-observed-adverse-effect level (NOAEL) of 8 mg/kg bw per day in a three-generation study of reproductive toxicity in rats and in a 1-year study in dogs (both of these studies having been evaluated at earlier meetings) and a safety factor of 100. The 1998 JMPR also concluded that an acute reference dose (ARfD) was not required because thiophanate-methyl is of low acute toxicity when administered orally or dermally and that the acute intake of residues is unlikely to present a risk to consumers.

The Meeting was asked by the Codex Committee on Pesticide Residues (CCPR) to reconsider the need for an ARfD for thiophanate-methyl. The present Joint Meeting therefore evaluated relevant original studies that had been considered by previous Meetings, and newly submitted studies on genotoxicity, reproductive toxicity, developmental toxicity and studies of acute neurotoxicity and short-term studies of neurotoxicity.

Evaluation for acute reference dose

1. Toxicological studies

1.1 Short-term studies of toxicity

Dogs

In a short-term study of toxicity conducted in compliance with the test guidelines of the United States Environmental Protection Agency (EPA), groups of four male and four female beagle dogs (age approximately 6 months at the start of the study) received gelatin capsules containing thiophanate-methyl (purity, 96.55%) at a dose of 0, 8, 40 or 200 mg/kg bw per day 1–2 h after the daily feed of 400 g, for 1 year. Physical observations, ophthalmic examinations, body weight and feed consumption measurements, haematology, biochemistry and urine analysis were performed on all animals before the test and at selected intervals during the treatment period. At sacrifice, selected organs were weighed, and all animals underwent extensive gross and histopathological examinations.

There were no deaths during the study. Tremors were seen in all dogs at 200 mg/kg bw per day approximately 2–4 h after treatment on one or more occasions during the initial 17 days of the study but not subsequently. The tremors were generally slight, except in one animal which had severe tremors that progressed to apparent tonic convulsions on three occasions (Table 1).

Table 1. Incidence of tremor in dogs given capsules containing thiophanate-methyl for 1 year

Dose (mg/kg bw per day)	Animal No.	Sex	Days of study on which tremors were observed	
			Slight tremors	Severe tremors/tonic convulsions
40	3604	Female	13	—
200	4101	Male	1, 7, 13	—
200	4102	Male	1	—
200	4103	Male	1, 4, 6, 7, 13	—
200	4104	Male	1, 7, 12, 13	—
200	4601	Female	1, 7	—
200	4602	Female	1	—
200	4603	Female	1, 2, 13, 16, 17	2, 16, 17
200	4604	Female	4	—

From Auletta (1992)

The mean body weights and body-weight gains of animals at 200 mg/kg bw per day were reduced throughout the study, and body-weight losses were noted in the first week of treatment. Two animals (male No. 4103, female No. 4604) exhibited further losses up to 0.6 kg at week 9 or 1.7 kg at week 15, respectively. Slight reductions in body-weight gain (about 81% of that of controls) were noted at 40 mg/kg bw per day. The feed consumption of dogs at the highest dose was generally lower than that of controls during the early months, but was close to the control value thereafter. Ophthalmological examinations and urine analyses showed no treatment-related effects. Haematological effects consisted of slight decreases in total erythrocyte counts and haemoglobin and erythrocyte volume fraction in males at 200 mg/kg bw per day (Table 2).

Table 2. Selected findings in dogs given capsules containing thiophanate-methyl for 1 year

Parameter	Dose (mg/kg bw per day)							
	Males				Females			
	0	8	40	200	0	8	40	200
Body weight (kg), month 12	13.0	13.7	12.1	10.4	10.7	12.4	9.9	8.5
Body-weight gain (kg), months 0–12	5.3	5.6	4.3	2.9	3.6	5.1	2.9	1.3*
Erythrocyte count (T/l), month 12	7.62	7.76	7.29	6.63**	7.61	7.23	7.35	7.25
Haemoglobin (g/dl), month 12	17.4	17.5	16.0	14.9**	16.9	16.1	16.8	16.6
Erythrocyte volume fraction, month 12	0.523	0.531	0.485	0.451**	0.518	0.489	0.518	0.514
Alkaline phosphatase (IU/l), month 12	50	35	76	150**	71	55	55	129
Cholesterol (mg/dl), month 12	166	170	216	236	169	232	210	298*
Albumin/globulin ratio, month 12	1.3	1.2	1.0*	0.9*	1.4	1.6	1.4	1.3
T3 (ng/ml)	1.09	1.19	1.19	1.06	1.14	1.37	1.40	1.70
T4 (µg/dl)	1.95	2.07	1.66	0.84*	1.96	2.23	2.42	2.07
TSH (ng/ml)	3.39	4.48	4.91	10.13	1.65	3.54	3.90	7.11
Liver weight (g)	307.7	317.4	344.2	364.2	272.5	317.0	290.6	288.8
Liver weight, relative (× 100)	2.42	2.31	2.88	3.52**	2.58	2.57	2.94	3.47*
Thyroid weight (g)	0.93	1.03	1.24	1.31	0.72	0.92	1.04*	1.00
Thyroid weight, relative (× 100 000)	7.36	7.63	10.43	12.67	6.79	7.49	10.46**	11.91**
Thyroid; follicular epithelial hypertrophy	0	0	0	4	0	0	2	3
Thyroid; follicular epithelial hyperplasia	0	0	0	1	0	0	0	1

From Auletta (1992)

* $p < 0.05$; ** $p < 0.01$

T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone.

The animals at the highest dose also had decreased serum alanine aminotransferase activity, increased alkaline phosphatase activity, increased cholesterol levels (also at the intermediate dose), and decreased albumin : globulin ratios (also at the intermediate dose), calcium (also at the intermediate dose), potassium and phosphorus concentrations. In females at 200 mg/kg bw per day, serum alanine aminotransferase activity was decreased, and alkaline phosphatase activity and cholesterol levels were increased. Thyroid function tests revealed decreased thyroxine concentrations in males at the intermediate and highest doses but no clear effects on triiodothyronine or thyroid-stimulating hormone. Abnormalities seen post mortem included increased liver weights in dogs at the highest dose and

increased thyroid weights in those at the intermediate and highest doses. Microscopic alterations attributed to treatment were limited to minimal to moderate hypertrophy and slight hyperplasia of the follicular epithelium of the thyroid in the dogs at the intermediate dose (hypertrophy in two females) and the highest dose (hypertrophy in four males and three females, and hyperplasia in one male and one female).

The NOAEL was 8 mg/kg bw per day, on the basis of increased thyroid weights and hypertrophy of the thyroid follicular epithelium at 40 mg/kg bw per day (Auletta, 1992).

In a short-term study of toxicity conducted in compliance with the test guidelines of the United States EPA, groups of four male and four female beagle dogs (aged approximately 7 months at the start of the study) were given gelatin capsules containing thiophanate-methyl (purity, 96.55%) at a dose of 0, 50, 200 or 800 mg/kg bw per day for 3 months, the last dose being lowered to 400 mg/kg bw per day on test day 50 because of severe toxicity. One male at the highest dose was sacrificed on day 41 because of severe toxicity; one male at 50 mg/kg bw per day died on day 36, but this death did not appear to be related to treatment. Dose-related clinical signs seen in animals at the highest dose and to a lesser extent at the intermediate dose included dehydration, thinness, and lethargy. Dose-related decreases in body weights and marked decreases in food consumption were seen at the intermediate and highest doses. There were no treatment-related ophthalmological findings. Slight anaemia, increased platelet counts and cholesterol levels, and decreases in serum alanine aminotransferase activity and albumin levels were seen at the intermediate and highest doses and increased activated partial thromboplastin time at the highest dose. Thyroid function tests revealed slightly decreased triiodothyronine levels in males at the highest dose and decreased triiodothyronine and thyroxine levels in females at the intermediate and highest doses; no clear effects on thyroid-stimulating hormone values were apparent. Urine analysis showed no treatment-related findings. At the intermediate and highest doses, the weights of the liver (in animals of each sex) and thyroid (males only) were increased. Gross examination post mortem revealed emaciation in one male at the intermediate dose and three at the highest dose. Dose-related histological alterations were seen in a number of organs. In thyroids, hypertrophy of the follicular epithelium was found in one, three and four males and one, two and four females at the lowest, intermediate and highest doses, respectively, with none in controls. The severity of the hypertrophy increased from minimal to marked with dose. Hyperplasia of the follicular epithelium was found in one male at the intermediate dose and in most animals at the highest dose. In two males at the highest dose in which both hypertrophy and hyperplasia were marked, a large decrease in the quantity of intrafollicular colloid was seen. In animals at the intermediate and highest doses, dose-related changes were found in the liver (reduction in vesiculation of the hepatocellular cytoplasm), gall-bladder (intracytoplasmic vacuoles), pancreas (atrophy of acinar cells owing to a decreased quantity of zymogen granules), spleen (lymphoid cell depletion), thymus (involution), prostate (atrophy), uterus (anestrus) and ovaries (inactive). No NOAEL was identified because of the presence of follicular cell hypertrophy in the thyroid of two dogs at the lowest dose (Auletta, 1991).

1.2 Genotoxicity

In a test for micronucleus formation performed in compliance with the test guidelines of the Organisation for Economic Co-operation and Development (OECD) and the United States EPA, groups of five male and five female B6D2F$_1$ mice were given thiophanate-methyl (purity, 97.28%) in 1% aqueus methylcellulose by oral gavage as single doses at 0, 500, 1000 or 2000 mg/kg bw, while a positive-control group was given carbendazim (purity, 98%) at a dose of 1000 mg/kg bw. Bone-marrow

smears were obtained from five males and five females in each group at 24 h and 48 h after dosing, with the exception that mice in the positive-control group were sampled at 24 h only. One smear from each animal was examined for the presence of micronuclei in 2000 immature erythrocytes. Doses were based on the results obtained in a preliminary study of toxicity in which plasma samples were taken to determine whether biologically significant concentrations of carbendazim can be expected after exposure to thiophanate-methyl in vivo (Table 3).

Table 3. Results of plasma analysis from a test for micronucleus formation in mice given single doses of thiophanate-methyl by gavage

Administered dose of thiophanate-methyl (mg/kg bw)	Sampling time (h)	Plasma concentration (ng/ml)[a]	
		Positive control (carbendazim)	Thiophanate-methyl
0	0	ND	ND
500	1	2033	5475
	3	411	2450
	6	559	1495
	12	28	55
	24	17	56
1000	1	3085	5370
	3	1483	3452
	6	651	691
	12	40	82
	24	< 5	< 5
2000	1	2776	7201
	3	1462	4311
	6	1578	4042
	12	567	2048
	24	47	76

From Proudlock (1999)

ND, not detected

[a] Mean values (from two males and two females).

Animals treated with thiophanate-methyl showed a small dose-related and statistically significant increase in the frequency of micronucleated immature erythrocytes at both sampling times, while a significant decrease in the proportion of immature erythrocytes was obtained in animals sampled at 24 h only. The positive control, carbendazim, produced large, significant increases in the frequency of micronucleated immature erythrocytes together with a significant decrease in the proportion of immature erythrocytes (Table 4).

Additional examinations (centromeric staining, size analysis of micronuclei) showed that carbendazim induced a high proportion (68%) of micronuclei containing centromers and a high proportion of large micronuclei (size, 40.1 units), while thiophanate-methyl produced an intermediate proportion of centromere-positive micronuclei (34%) and micronuclei of intermediate size (31.9 units). The clastogenic compound mitomycin C produced a low proportion of centromere-positive micronuclei (24%) and mainly small micronuclei (size, 31.9 units).

Table 4. Results of bone-marrow smear analysis from a test for micronucleus formation in mice given single doses of thiophanate-methyl by gavage

Sampling time (h)	Treatment	Dose (mg/kg bw)	Proportion of immature erythrocytes[a]	Micronuclei in immature erythrocytes[b]	Micronuclei in mature erythrocytes[c]
24	Vehicle control	0	45	1.3	0.3
	Thiophanate-methyl	500	46	4.2**	0.3
		1000	44	3.8**	0.9
		2000	41*	6.3**	1.2
	Positive control (carbendazim)	1000	38**	24.5**	2.2
48	Vehicle control	0	43	1.2	1.3
	Thiophanate-methyl	500	43	3.1**	0.3
		1000	45	5.0**	0.0
		2000	44	5.6**	1.6

From Proudlock (1999)

* $p < 0.01$; ** $p < 0.001$

[a] Proportion of immature erythrocytes [% immature erythrocytes / (immature erythrocytes + mature erythrocytes)]

[b] No. of micronucleated cells per 2000 immature erythrocytes examined.

[c] No. of micronucleated cells per 2000 mature erythrocytes examined.

1.3 Reproductive toxicity

(a) Multigeneration studies

In a dose range-finding study for a two-generation study of reproductive toxicity, groups of 7 male and 14 female Sprague-Dawley Crl:CD(SD)BR rats were fed diets containing thiophanate-methyl (purity, 95.93%) at a concentration of 0, 75, 200, 1200 or 6000 ppm from the start of the treatment until necropsy. After a pre-mating treatment period of 14 days, each male was mated with two females in the same dose group for a maximum of 3 weeks and the females were allowed to litter and to rear their offspring to weaning. Determinations for clinical signs, body weights, feed consumption, mating, fertility and litter size were performed during the study. Offspring were examined for sex ratio, viability, clinical signs and body-weight gain. The males were sacrificed shortly after the mating period and the dams and pups after weaning. Gross necropsy evaluations were performed on all adults and pups and liver and thyroid of parental animals were weighed. Dietary analyses revealed satisfactory test substance stability and concentration. The mean daily intakes at 75, 200, 1200 and 6000 ppm in the pre-mating period were 5.1–5.4, 13.6–14.5, 82.1–85.1 and 361.3–375.3 mg/kg bw per day in males and 6.3–6.5, 16.4–17.5, 101.9–102.5 and 474.2–480.8 mg/kg bw per day in females, respectively.

No mortalities or treatment-related clinical findings were observed during the study. Feed consumption at 6000 ppm was markedly reduced in males before mating, while in females it was slightly to moderately reduced during the entire study. Body-weight gain was slightly or markedly reduced in males at 1200 and 6000 ppm before and during mating and in females at 6000 ppm before and during mating and during gestation. Thyroid weights were moderately to markedly increased at 1200 and 6000 ppm in males and females, while liver weights were moderately increased in females at 1200 and 6000 ppm (Table 5). There were no treatment-related effects on fertility or reproductive performance and on the development of pups.

The NOAEL for parental toxicity was 200 ppm (equal to 13.6–14.5 mg/kg bw per day in males and 16.4–17.5 mg/kg bw per day in females, respectively), on the basis of reduced body-weight gain in males, increased thyroid weights in males and females and increased liver weights in females at 1200 ppm and above. The NOAEL for reproductive toxicity was 6000 ppm (equal to 361.3–375.3 mg/kg bw per day in males and 474.2–480.8 mg/kg bw per day in females, respectively), the highest dose tested (Müller, 1992).

Table 5. Selected findings in the parental generation of rats given diets containing thiophanate-methyl in a range-finding study of reproductive toxicity

Parameter	Dietary concentration (ppm)				
	0	75	200	1200	6000
Males					
Feed consumption (g/day), pre-mating, days 11–15	33.4	32.0	32.6	31.8	26.6**
Body-weight gain (g), pre-mating, days 1–15	52.6	54.8	60.5	47.5	28.8*
Body weight (g), termination	524.4	522.0	516.1	508.2	475.8*
Thyroid weight (mg)	25.6	29.0	24.6	31.4	49.3*
Liver weight (g)	22.3	24.6	22.1	24.3	24.0
Females					
Feed consumption (g/day), pre-mating, days 11–15	22.3	22.7	21.9	22.0	19.9*
Feed consumption (g/day), gestation, days 0–20	26.4	26.9	27.6	27.3	23.0**
Feed consumption (g/day), lactation, days 0–21	55.8	47.3	57.2	59.5	46.5
Body-weight gain (g), pre-mating, days 1–15	30.2	31.5	30.0	29.5	18.1**
Body-weight gain (g), gestation, days 0–20	153.7	156.9	160.5	161.3	131.7*
Body weight (g), termination	325.2	332.5	337.2	335.9	312.2
Thyroid weight (mg)	20.2	23.2	21.8	29.8*	48.4*
Liver weight (g)	17.5	17.1	19.0	20.9*	20.9*

From Müller (1992)

* $p \leq 0.05$; ** $p \leq 0.01$

(b) Developmental toxicity

Mice

In a study of prenatal developmental toxicity, groups of 20 pregnant ICR mice were given thiophanate-methyl (purity, not reported) in 5% gum arabic aqueous solution by gavage at a dose of 0, 40, 200, 500 or 1000 mg/kg bw per day on days 1–15 of gestation (the day after overnight mating being considered to be day 1). The mice were observed for clinical signs and were weighed on days 1, 15 and 19. The animals were sacrificed on day 19 of gestation, and the numbers of implantation sites and live and dead fetuses were counted. The live fetuses were examined for sex, body weight, and gross external, visceral, and skeletal alterations.

No signs of toxicity were observed in the dams, throughout the study period. There were no significant differences in the number of implantation sites, fetal body weights or the incidence of

malformations between the control and the dosed groups. At 1000 mg/kg bw per day, the number of live fetuses was significantly decreased compared with the controls (Table 6).

Table 6. Selected maternal and reproductive findings in a study of prenatal developmental toxicity in mice given thiophanate-methyl by gavage

Parameter	Dose (mg/kg bw per day)				
	0	40	200	500	1000
Body weight (g), day 19	56.5	54.5	51.8	52.4	52.8
Total No. of implantations	244	225	221	233	225
Mean No. of implantations	12.20	11.25	11.05	11.65	11.25
Total No. of live fetuses	218	204	202	212	195*
Mean No. of live fetuses	10.90	10.20	10.10	10.60	9.70*
Mean fetal weight, live fetuses (g)	1.13	1.12	1.18	1.14	1.10
Malformed live fetuses (No. / %)[a]	2/0.77	1/0.44	2/0.90	1/0.43	2/0.89
Dead fetuses; immature (No. / %)	17/6.97	9/4.00	10/4.53	13/6.45	12/5.33
Dead fetuses; resorbed (No. / %)	9/3.69	12/5.33	9/4.07	6/2.57	18/8.00

From Noguchi & Hashimoto (1970)

* $p \leq 0.05$

[a] Fetuses with cleft palate.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 500 mg/kg bw per day, on the basis of decreased number of live fetuses at 1000 mg/kg bw per day (Noguchi & Hashimoto, 1970).

Rabbits

In a dose range-finding study for a prenatal developmental study of toxicity, groups of 6 presumed pregnant New Zealand white [Hra:(NZW)SPF] rabbits were given technical-grade thiophanate-methyl at a dose of 0, 5, 10, 20, 40 or 80 mg/kg bw per day in 1% methylcellulose by gavage on days 6 to 28 of gestation. The rabbits were sacrificed on day 29 of gestation. The does were observed for viability, clinical signs, body weight, food consumption and the number of corpora lutea, and the thoracic, abdominal, and pelvic viscera were examined. Their uteri were excised and examined for the number and distribution of implantation sites, live and dead fetuses and early and late resorptions. The fetuses were examined for sex and gross external alterations, and the body weights were recorded. Fetuses from the control, lowest- and highest-dose groups were also examined for visceral and skeletal alterations.

At 80 mg/kg bw per day, there were two abortions on days 23 and 24, respectively, which were considered to be treatment-related. Regarding clinical observations, faecal output, feed consumption and body-weight gain were reduced over the entire treatment period at 40 mg/kg bw per day and above, while at 20 mg/kg bw per day, feed consumption and body-weight gain were reduced only on days 6 to 9 of gestation (Table 7). At 80 mg/kg bw per day, there was an increase in the number of early resorptions and a concomitant decrease in litter size. In the fetuses examined for visceral and skeletal alterations, the number of thoracic rib pairs was increased at 80 mg/kg bw per day, with associated increases and decreases in the averages for thoracic and lumbar vertebrae, respectively (York, 1997a).

Table 7. Selected maternal and reproductive findings in a range-finding study of prenatal developmental toxicity in rabbits given thiophanate-methyl by gavage

Parameter	Dose (mg/kg bw per day)					
	0	5	10	20	40	80
Body-weight gain (kg), days 6–9	0.05	0.07	0.08	–0.04	–0.18	–0.24
Body-weight gain (kg), days 6–29	0.43	0.26	0.26	0.30	0.12	0.03
Feed consumption (g/day), days 6–9	190.1	172.9	185.1	121.1	65.5	11.1
Feed consumption (g/day), days 6–29	167.7	144.4	144.6	137.6	101.8	81.2
No. of dams pregnant/with live fetuses	6/6	6/5	5/5	5/5	6/6	6/3
No. of dams aborted/found dead	0/0	0/1	0/0	0/0	0/0	2/0
Mean No. of corpora lutea/implantations	9.7/8.8	7.8/7.4	9.2/8.8	9.6/9.0	9.0/8.3	9.8/9.0
Mean litter size	8.8	7.2	8.2	8.6	8.3	5.2
No. of live/dead fetuses	53/0	36/0	41/0	43/0	50/0	21/0
No./mean No. of early resorptions	0	1/0.2	2/0.4	1/0.2	0	13/3.2
No./mean No. of late resorptions	0	0	1/0.2	1/0.2	0	2/0.5
No./% of does with any resorption	0	1/20	2/40	2/40	0	3/75
Mean % resorbed conceptuses per litter	0	3.3	5.5	4.7	0	10.3
Mean live fetal body weights (g)	39.61	40.91	41.37	40.19	40.27	40.02
Litters/fetuses with any alterations	4/7	3/4	1/1	0	0	1/1
% Fetuses with any alteration per litter	12.7	8.1	1.5	0	0	6.7
Mean No. of ribs (pairs)	12.45	12.24	NE	NE	NE	12.87
Mean No. of cervical vertebrae	7.00	7.00	NE	NE	NE	7.00
Mean No. of thoracic vertebrae	12.48	12.34	NE	NE	NE	12.90
Mean No. of lumbar vertebrae	6.48	6.66	NE	NE	NE	6.10
Mean No. of sacral vertebrae	3.00	3.00	NE	NE	NE	3.00
Mean No. of caudal vertebrae	16.75	16.86	NE	NE	NE	17.10

From York (1997a)

NE, not examined.

In a study of prenatal developmental toxicity, groups of 20 naturally bred New Zealand white [Hra:(NZW)SPF] rabbits were given technical-grade thiophanate-methyl (purity, 97.28–97.57%) at a dose of 0, 5, 10, 20 or 40 mg/kg bw per day in 1% methylcellulose by gavage on days 6–28 of gestation, the day of mating being considered to be day 0. The rabbits were sacrificed on day 29 of gestation. The does were observed for viability, clinical signs, body weight, food consumption, and the number of corpora lutea and the thoracic, abdominal, and pelvic viscera were examined. Their uteri were excised and examined for the number and distribution of implantation sites, live and dead fetuses and early and late resorptions. The fetuses were observed for sex, body weight and gross external, visceral, brain and skeletal alterations.

At a dose of 20 mg/kg bw per day, there was a transient but significant reduction in maternal body-weight gain and statistically significant reduced absolute and relative feed consumption. At a dose of 40 mg/kg bw per day, faecal output was reduced, in conjunction with significantly reduced maternal body-weight gain and absolute and relative feed consumption; however, body-weight gain and food consumption recovered after the initial week of dosing. It is likely that the observed reduction in feed consumption during this period contributed to the observed decreases in body weight, body-weight gain and faecal output. There appeared to be an increased incidence of resorptions in rabbits treated with 40 mg/kg bw per day, the numbers of litters with ≥ 20% resorptions per litter being 2 of 20 in the controls, 2 of 17 at 5 mg/kg bw per day, 2 of 18 at 10 mg/kg bw per day, 0 of 17 at 20 mg/kg bw per day, and 5 of 20 at 40 mg/kg bw per day. The historical control range for dead or resorbed conceptuses per litter was 0–18.3%. In the fetuses, the dose of 40 mg/kg bw per day caused a significant increase in the average for thoracic ribs (supernumerary ribs), with associated significant increases and decreases in the averages for thoracic and lumbar vertebrae, respectively. In addition, fetal body weights were slightly reduced (about 9%; not statistically significant) at the dose of 40 mg/kg bw per day (Table 8).

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced feed consumption and reduced body-weight gain at 20 mg/kg bw per day and above. The NOAEL for developmental effects was 20 mg/kg bw per day, on the basis of increased incidence of supernumerary thoracic ribs and decreased fetal weights at 40 mg/kg bw per day, the highest dose tested. Thiophanate-methyl was not selectively toxic to embryo or fetal viability, growth or morphology and was not teratogenic (York, 1997b).

Table 8. Selected maternal and reproductive findings in a study of prenatal developmental toxicity in rabbits given thiophanate-methyl by gavage

Parameter	Dose (mg/kg bw per day)				
	0	5	10	20	40
Body-weight gain (kg), days 6–9	0.08	0.07	0.09	0.06	–0.11**
Body-weight gain (kg), days 12–15	0.09	0.11	0.08	0.04*	0.03**
Body-weight gain (kg), days 6–29	0.41	0.44	0.44	0.36[c]	0.10**
Feed consumption (g/day), days 6–9	180.7	175.0	182.4	152.3	58.8**
Feed consumption (g/day), days 6–29	159.0	160.6	160.9	140.7*	89.3**
No. of dams pregnant/with live fetuses	20/19[a]	17/17	19/18[b]	17/17	20/19[a]
Mean No. of corpora lutea/implantations	10.3/9.3	9.4/8.9	9.9/9.7	8.6/7.6	9.9/9.2

Mean litter size	8.8	8.4	9.1	7.4	8.4
No. of live/dead fetuses	168/0	141/2	164/0	125/0	160/0
No./mean No. of early resorptions	7/0.4	5/0.3	5/0.3	3/0.2	7/0.4
No./mean No. of late resorptions	2/0.1	3/0.2	5/0.3	1/0.0	7/0.4
No./% of does with any resorption	7/36.8	7/41.2	6/33.3	4/23.5	9/47.4
No. of does with ≥ 20% resorptions	2	2	2	0	5
No. of litters/fetuses evaluated	19/168	17/143	18/164	16/115[c]	19/160
Mean live fetal body weights (g)	44.78	43.05	42.68	45.56	40.64
Litters/fetuses with any alterations	11/14	12/23**	8/11	13/21**	10/16
% Fetuses with any alteration per litter	8.9	15.4	7.5	18.6*	10.0
Mean No. of ribs (pairs)[a]	12.45	12.44	12.45	12.58	12.85**
Mean No. of cervical vertebrae	7.00	7.00	7.00	7.00	7.00
Mean No. of thoracic vertebrae[b]	12.50	12.52	12.53	12.68	12.89**
Mean No. of lumbar vertebrae[c]	6.48	6.47	6.46	6.32	6.09**
Mean No. of sacral vertebrae	3.00	3.00	3.00	3.00	3.00
Mean No. of caudal vertebrae	16.96	17.02	17.04	16.98	17.06

From York (1997b)

* $p \leq 0.05$; ** $p \leq 0.01$

[a] Historical control range: ribs: 12.34–12.67; thoracic vertebrae: 12.38–12.70; lumbar vertebrae: 6.30–6.61

[a] Excludes values for does that had litters consisting of all early resorptions.

[b] Excludes values for doe which delivered on day 12 of presumed gestation (mating date incorrectly identified).

[c] Excludes values for doe which prematurely delivered on day 29 of gestation.

1.4 Special studies: neurotoxicity

In a study of acute neurotoxicity that complied with the test guidelines of the United States EPA and OECD, groups of 10 male and 10 female Crl:CD(SD)IGS BR VAF Plus rats were given thiophanate-methyl (purity, 99.7%) at a dose of 0, 500, 1000 or 2000 mg/kg bw as a suspension in aqueous 0.5% (w/v) methylcellulose by gavage once on day 1 of the study . In the extension part of the study, which was conducted to evaluate additional dosages for several parameters affected in the main study, groups of 10 male and 10 female rats were given thiophanate-methyl at a dose of 0, 50, 125, 500 or 2000 mg/kg bw by gavage on day 1 of the study. The dosage volume was 10 ml/kg in both study phases.

In the main study, viabilities, clinical observations, body weights, feed consumption values, functional observational battery (FOB) evaluations (which included detailed clinical observations) and motor activity evaluations were recorded. Rats were sacrificed on day 15, administered a

combination of heparin and an anaesthetic and perfused in situ with neutral buffered 10% formalin. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Brain weights were recorded for all rats after additional fixation of the tissue. Five rats of each sex per group were selected for neurohistological examination; and the tissues from rats selected in the control groups and group at the highest dose were processed for histological examination. In the study extension, viabilities, clinical observations, body weights, feed consumption values, two components of the FOB (behaviour in the home cage and measurement of landing foot splay) and motor activity evaluations (female rats only) were evaluated. These rats were sacrificed on day 3 of the study, and carcasses were discarded without further evaluation.

All rats survived until scheduled necropsy on day 15 (main study) or day 3 (extension study). None of the clinical signs observed in these rats were considered to be related to the test substance. Body weights and feed consumption values were unaffected at a dose of 50 or 125 mg/kg bw in the extension study. Transient reductions in body-weight gains or body-weight losses along with corresponding reductions in absolute (g/day) and relative (g/kg per day) feed consumption occurred on the day after dosage in both sexes given thiophanate-methyl at 500 mg/kg bw and higher in the main study, the extension study or both. These changes were followed by increases in body-weight gain on the subsequent day.

In the FOB conducted 2 h after dosing in the main study, both male and female rats given thiophanate-methyl at 500, 1000 or 2000 mg/kg bw had significantly reduced values for landing foot splay (Table 9). The numbers of male rats in these groups that appeared to be sleeping when examined in the home cage also were significantly reduced, and the numbers showing other normal patterns (e.g. appeared awake and immobile or showed normal movement) were increased when compared with the control group. Female rats at 500 mg/kg bw and higher also had significantly reduced motor activity during the test that followed the FOB examination on the day of dosing. There were neither statistically significant nor exceptional differences among the groups in the measures of the functional observational battery or in the motor activity test on the day before dosage and 7 days and 14 days after dosing. No gross lesions were observed at necropsy. Brain weights and the ratio of brain weights to terminal body weights were comparable among the groups. No lesions related to the test substance were observed in the microscopic examination of the neural and muscle tissues.

The extension study evaluated home cage behaviour and landing foot splay in both sexes and motor activity in females dosing dosage and 2 h after dosing. Landing foot-splay values were significantly reduced in both sexes at 50 mg/kg bw and higher (Table 9). The variations in normal home-cage behaviour and reduced motor activity observed in the main study were not observed at 50 and 125 mg/kg bw and were not replicated at 500 and 2000 mg/kg bw in the study extension. These changes were considered to be incidental events because they were noted only in one sex in the main study and not reproduced in the extension study. This conclusion is also supported by the evaluations in the short-term study of neurotoxicity in which there were no adverse changes in the FOB, including the home-cage observation and motor activity tests at the highest dietary concentration of 2500 ppm at 2, 4, 8 and 13 weeks of exposure.

The toxicological significance of the transient decreases in landing foot splay observed in this study is questionable and considered inappropriate for setting a NOAEL. The biological significance of decreased landing foot splay in the absence of apparent changes in gait, posture or other behavioural responses such as air righting has not been specified in previous research. In addition, the variability observed within the pairs of measurements obtained from individual rats as well as variability among the averages for rats in the control group in this study and across other studies were comparable to the differences between the control groups and the groups given thiophanate-methyl. This conclusion is also supported by the evaluations in the short-term study of neurotoxicity in which there were no

adverse changes in the FOB, including the landing foot-splay measurements, at the highest dietary concentration of 2500 ppm (equal to 149.6 mg/kg bw per day in males and 166.3 mg/kg bw per day in females) at 2, 4, 8 and 13 weeks of exposure.

Table 9. Landing foot splay (cm) in rats given a single dose of thiophanate-methyl by gavage in a study of acute neurotoxicity

Sex	Study[a]	Time-point	Dose (mg/kg bw)					
			0	50	125	500	1000	2000
Males	Main study	Before dosing	7.0	NE	NE	7.1	6.6	7.4
		2 h after dosing	6.6	NE	NE	5.2*	4.2**	4.7**
		7 days after dosing	6.5	NE	NE	6.9	6.6	7.0
		14 days after dosing	6.4	NE	NE	6.8	6.1	6.4
	Study extension	Before dosing	8.4	8.9	8.2	7.6	NE	8.2
		2 h after dosing	8.9	7.0**	6.5**	5.2**	NE	6.1**
Females	Main study	Before dosing	6.2	NE	NE	6.5	6.4	7.2
		2 h after dosing	5.6	NE	NE	3.9**	4.2**	4.4**
		7 days after dosing	5.5	NE	NE	5.1	5.6	5.8
		14 days after dosing	5.3	NE	NE	4.8	5.5	5.9
	Study extension	Before dosing	8.4	8.0	8.3	7.4	NE	7.9
		2 h after dosing	8.4	6.8*	6.0**	6.0**	NE	5.6**

From Foss (2005a)

* $p \leq 0.05$; ** $p \leq 0.01$

NE, not examined.

[a] Data for historical controls: males 6.5 ± 1.7 cm and 8.7 ± 1.2 cm; females, 6.3 ± 1.2 cm and 5.7 ± 1.7 cm.

The NOAEL for general toxicity was 125 mg/kg bw on the basis of transient reductions in body-weight gains (including body-weight losses) and feed consumption at 500 mg/kg bw and above. The NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested. The toxicological significance of the transient decreases in landing foot splay observed at all doses was questionable and was considered to be inappropriate for identifying a NOAEL for neurotoxicity (Foss, 2005a).

In a short-term study of neurotoxicity conducted in compliance with the test guidelines of the United States EPA and OECD, groups of 10 male and 10 female Crl:CD(SD)IGS BR VAF Plus rats were fed diets containing thiophanate-methyl (purity, 99.7%) at a concentration of 0, 100, 500 or 2500 ppm for 13 weeks (91 days). Viability, clinical observations, body-weights, feed consumption, functional observational battery (FOB) evaluations (which included detailed clinical observations) and motor activity evaluations were recorded. At termination rats were administered a combination of heparin and an anaesthetic and perfused in situ with neutral buffered 10% formalin. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Liver and individual kidney weights were recorded at necropsy, and brain weights were recorded after additional fixation of the tissue. Five rats of each sex per group were selected for neurohistological examination; and the tissues from rats selected in the control group and the group receiving the highest dietary concentration were evaluated.

Table 10. Selected findings from a short-term study of neurotoxicity in rats given diets containing thiophanate-methyl for 13 weeks

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	100	500	2500	0	100	500	2500
Body weight (g), day 91	540.6	532.8	544.5	519.9	309.9	290.1	296.3	280.2
Body-weight gain (g), days 1–91	329.1	323.6	335.0	309.7	140.0	121.7*	127.3	110.6**
Feed consumption (g/day)	24.7	25.1	24.9	23.8	18.7	16.8**	17.6	15.9**
Forelimb grip, maximum (g), week 2	284.5	335.5	335.5	340.0	291.5	201.0**	264.0	222.5*
Forelimb grip, average (g), week 2	257.2	295.8	303.2	289.2	259.8	177.8**	235.0	204.5*
Hindlimb grip, maximum (g) week 2	296.5	338.5	366.5	291.5	279.5	202.0**	206.5*	213.0*
Hindlimb grip, average (g), week 2	259.2	315.5	325.2	262.0	236.8	184.5*	187.8*	187.2*
Liver								
Absolute weight (g)	22.95	20.75	23.91	27.66*	12.95	11.80	12.88	13.92
Relative weight (g/100 g bw)	4.24	3.89	4.37	5.32**	4.19	4.06	4.34	4.97**
Thyroid								
Absolute weight (mg)	29	30	34	57**	28	21	26	35
Relative weight (mg/100 g bw)	5.45	5.72	6.25	10.97**	8.87	7.03	8.84	12.45**

From Foss (2005b)

* $p \leq 0.05$; ** $p \leq 0.01$

Mean intakes of test substance were 6.2, 30.3 and 149.6 mg/kg bw per day for males and 6.8, 34.9 and 166.3 mg/kg bw per day for females at 100, 500 and 2500 ppm, respectively. None of the clinical signs observed in the daily examinations were considered to be related to the test substance. Body weights, body-weight changes and absolute and relative feed consumption values were significantly decreased at 2500 ppm in females, while these values were unaffected in males (Table 10). Other than the body weights in the female rats, none of the parameters evaluated in the FOB and motor activity test sessions were affected by the dietary concentrations of thiophanate-methyl administered in this study. The decreases in the forelimb and hindlimb grip in females at 100 ppm and higher during week 2 of exposure were considered to be incidental events unrelated to the test substance, because the differences were not dose-dependent and there were no effects on other FOB parameters that might be affected by muscle weakness. There were no gross lesions in either sex, but the absolute weights of the liver and thyroid as well as ratios of these weights to terminal body weight and to brain weight were significantly increased at 2500 ppm in male rats; the ratios of these weights to terminal body weight were also significantly increased in female rats at this concentration. No microscopic lesions were observed in the neural or muscle tissues of the rats in the control group or at 2500 ppm.

The NOAEL for general toxicity was 500 ppm (equal to 30.3 and 34.9 mg/kg bw per day in males and females, respectively) on the basis of decreased body weights and feed consumption in females and increased liver and thyroid weights in both sexes at 2500 ppm. The NOAEL for neurotoxicity was 2500 ppm (equal to 149.6 and 166.3 mg/kg bw per day in males and females, respectively), the highest dose tested (Foss, 2005b).

Comments

Toxicological data

Thiophanate-methyl has low acute toxicity: the oral median lethal dose (LD_{50}) was 6640–7500 mg/kg bw in rats and 3400–3514 mg/kg bw in mice. The clinical signs of toxicity after single high (near-lethal) doses included whole body tremors at 1–2 h after dosing, which progressed to tonic and clonic convulsions.

In a 1-year study of toxicity in dogs given capsules containing thiophanate-methyl, slight tremors were observed in all eight animals approximately 2–4 h after administration of the highest dose of 200 mg/kg bw per day on one to five occasions during the initial 17 days of the study. One dog exhibited severe tremors that progressed to tonic convulsions on three occasions. The NOAEL was 8 mg/kg bw per day on the basis of increased thyroid weights and hypertrophy of the thyroid follicular epithelium at 40 mg/kg bw per day and above.

In a 3-month study of toxicity in dogs given capsules containing thiophanate-methyl at doses of up to 800 mg/kg bw per day, dose-related clinical signs including dehydration, thinness and lethargy were seen in animals at the highest dose and to a lesser extent at the intermediate dose (200 mg/kg bw per day). No tremors were seen up to the highest dose tested. A NOAEL could not be identified in this study because of the presence of follicular-cell hypertrophy in the thyroid of two dogs at 50 mg/kg bw per day, the lowest dose tested.

Thiophanate-methyl has been adequately tested in a range of assays for genotoxicity. Thiophanate-methyl does not cause gene mutations or structural chromosomal aberrations; however, it causes changes in chromosome number (aneuploidy) both in vitro and in vivo. Induction of micronucleus formation in mice was seen after single highest doses (500 mg/kg bw and above), but the response was weak when compared with that for the main metabolite of thiophanate-methyl, carbendazim, which is considered to be responsible for the observed effect. The mechanism by which aneuploidy is induced by carbendazim is clearly understood and there is a clear threshold for this effect.

The Meeting concluded that the genotoxic effect of thiophanate-methyl is a threshold phenomenon and is related to the production of carbendazim.

On the basis of evaluations from previous Meetings, there was no adverse effect on fertility and reproductive performance in a recent two-generation study of reproduction toxicity using doses of up to 2000 ppm, equal to 147.1 and 164.3 mg/kg bw per day in males and females, respectively.

The developmental toxicity potential of thiophanate-methyl had been investigated in mice, rats and rabbits. From evaluations made at previous Meetings, the NOAEL for developmental toxicity in mice was 500 mg/kg bw per day, on the basis of decreased number of live fetuses at 1000 mg/kg bw per day, while no maternal toxicity was observed at this dose. In rats, there was no evidence of developmental toxicity at doses of up to 1000 mg/kg bw per day, but maternal toxicity (reduced body-weight gain) was seen at this dose.

In a study of prenatal developmental toxicity in rabbits, which had not been evaluated previously, the NOAEL for developmental effects was 20 mg/kg bw per day on the basis of increased incidence of supernumerary thoracic ribs and decreased fetal weights at 40 mg/kg bw per day, the highest dose tested. The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced feed consumption and reduced body-weight gain at 20 mg/kg bw per day and above.

Thiophanate-methyl was not selectively toxic to embryo or fetal development in rats and rabbits and was not teratogenic.

In a study of acute neurotoxicity in rats, the NOAEL for general toxicity was 125 mg/kg bw on the basis of transient reductions in body-weight gains (including body-weight losses) and feed consumption at 500 mg/kg bw and above. The NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested.

In a short-term study of neurotoxicity in rats, the NOAEL for general toxicity was 500 ppm (equal to 30.3 and 34.9 mg/kg bw per day in males and females, respectively) on the basis of decreased body weights and feed consumption in females and increased liver and thyroid weights in both sexes at 2500 ppm. No neurohistological changes were seen at 2500 ppm. The NOAEL for neurotoxicity was 2500 ppm (equal to 149.6 and 166.3 mg/kg bw per day in males and females, respectively), the highest dose tested.

Toxicological evaluation

The Meeting considered whether the establishment of an ARfD was necessary. The initial, transient clinical signs (tremors) that were seen in a 1-year study in dogs given thiophanate-methyl at a dose of 200 mg/kg bw per day were not observed in a 3-month study in dogs given thiophanate-methyl at doses of up to 800 mg/kg bw per day. Therefore the Joint Meeting considered that the tremors were not attributable to a toxicological effect of the test substance.

The developmental effects that had been observed in rabbits at 40 mg/kg bw per day were not considered to be elicited by a single exposure.

The Meeting concluded that it was not necessary to establish an ARfD 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.

The Meeting noted that the use of thiophanate-methyl on crops may give rise to residues of carbendazim, although thiophanate-methyl can also be detected as part of the residue to which consumers of treated produce are exposed. Since the toxicity of thiophanate-methyl is qualitatively and quantitatively (when corrected for relative molecular mass) different from that of carbendazim, and since the ARfD for carbendazim is lower than that which would be derived from data on thiophanate-methyl, the Joint Meeting concluded that the intake of residues in food should initially be compared with the ARfD for carbendazim. If further refinement of the risk assessment is necessary, the different components of the residue (carbendazim and thiophanate-methyl) could be considered separately.

Estimate of acute reference dose

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposures

References

Auletta, C.S. (1991) A subchronic (3-month) oral toxicity study in the dog via capsule administration with thiophanate-methyl. Unpublished report No. RD-9119, project No. 89-3525 from Bio/dynamics Inc., East Millstone, New Jersey, USA. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

Auletta, C.S. (1992) A chronic (1-year) oral toxicity study in the dog via capsule administration with thiophanate-methyl. Unpublished report No. RD-9207, project No. 89-3526 from Bio/dynamics Inc., East Millstone, New Jersey, USA. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

Foss, J.A. (2005a) Oral (gavage) acute neurotoxicity study of thiophanate-methyl in rats. Unpublished report No. RD-00828, project No. 914-006 from CR-DDS Argus Division, Horsham, Pennsylvania, USA. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

Foss, J.A. (2005b) Oral (diet) subchronic neurotoxicity study of thiophanate-methyl in rats. Unpublished report No. RD-00634, project No. 914-007 from CR-DDS Argus Division, Horsham, Pennsylvania, USA. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

Müller, W. (1992) Dose range-finding study for a two generation oral (dietary administration) reproduction toxicity study in the rat. Unpublished report No. RD-9227, project No. 683-003 from Hazleton Laboratories Deutschland GmbH, Münster, Deutschland. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

Noguchi, T. & Hashimoto, Y. (1970) Toxicological evaluation of thiophanate-methyl (IV) studies on the teratogenic effect of thiophanate-methyl upon fetus of ICR strain of mice. Unpublished report No. RD-73055 from Nisso Institute for Life Science, Oiso, Kanagawa, Japan. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

Proudlock, R.J. (1999) Thiophanate-methyl mouse micronucleus test. Unpublished report No. NOD 024/983714 from Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England. Submitted to WHO by Nippon Soda Co., Ltd. Tokyo, Japan.

York, R.G. (1997a) Oral (stomach tube and dietary) dosage-range developmental toxicity study of thiophanate-methyl in rabbits. Unpublished report No. RD-9769, project No. 914-002P from Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Submitted to WHO by Nippon Soda Co., Ltd. Tokyo, Japan.

York, R.G. (1997b) Oral (stomach tube) developmental toxicity study of thiophanate-methyl in rabbits. Unpublished report No. RD-9770, project No. 914-002 from Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Submitted to WHO by Nippon Soda Co., Ltd., Tokyo, Japan.