

## THIRAM

First draft prepared by J.-J. Larsen  
National Food Agency, Ministry of Health  
Søborg, Denmark

### EXPLANATION

Thiram, a dimethyl dithiocarbamate fungicide, was evaluated by the Joint Meeting several times between 1963 and 1987 (Annex 1, references 2, 4, 8, 14, 22, 28, 34, 44 and 50). A temporary ADI of 0-0.005 mg/kg bw, allocated in 1974, was extended in 1977 and 1980. The temporary ADI was withdrawn in 1985 because of the inadequacy of the total data base. The studies available to the 1987 Joint Meeting were not adequate for estimating an ADI. A complete data base on thiram has been generated since the previous evaluation, and was evaluated at the present Meeting.

### EVALUATION FOR ACCEPTABLE DAILY INTAKE

#### BIOLOGICAL DATA

##### Biochemical aspects

##### **Absorption, distribution, and excretion**

A single oral dose of  $^{14}\text{C}$ -thiram was administered to male and female Charles River CD random bred, VAF Plus (SPF) rats to determine its absorption, excretion and final distribution. Unlabelled thiram (purity 98.5%) and  $^{14}\text{C}$ -thiram (radiochemical purity 100%) dissolved in PEG-400 solution was administered by gavage at 125 mg/kg bw (dosage volume 4.95 ml/kg, concentration 25.24 mg/ml, specific activity  $1.748 \times 10^7$  dpm/ml ( $\pm 0.006 \times 10^7$  SD)), to 5 rats/sex and at 1.9 mg/kg bw (dosage volume 4.75 ml/kg, concentration 0.4 mg/ml, specific activity  $1.721 \times 10^7$  dpm/ml ( $\pm 0.003 \times 10^7$  SD)), to 5 rats/sex (weight range 180-250 g). PEG-400 solution was administered to one rat/sex as controls. Urine and faeces were collected for 7 days. The rats were then necropsied and tissues were collected. Homogenates (20%) were prepared from the tissues and faecal material and were oxidized. Radioactivity was determined by liquid scintillation counting.

Only 32% of the administered dose was recovered, mainly from the urine (25%). About 3% was recovered from the various organs. Blood, bone and liver contained significant quantities of the test material or its metabolites. Only 3% of the administered dose was recovered in the faeces. Dose level or sex did not affect total recovery. Approximately 70% of the administered thiram, not recovered, may

have been metabolized to CO<sub>2</sub> or other volatiles in the expired air or by bacterial action in the faeces or urine during the intervals between collections (Gay, 1987).

Following 14 days pretreatment to 5 Taconic farm Sprague-Dawley rats/sex (males 6-9 weeks old, 227-250 g; females 9-10 weeks old, 197-220 g) at a dose level of 2 mg thiram/kg bw/day, a single dose of <sup>14</sup>C-thiram/kg bw (radiochemical purity > 98%, specific activity 15.5 mCi/mmol) was administered. Doses were administered as a suspension in PEG-400 (5 ml/kg bw). The stability of labelled and unlabelled thiram was satisfactory. The percentages of <sup>14</sup>C-thiram-derived radioactivity were determined in urine, faeces and expired air at intervals up to 96 hr, and the concentrations and percentages were determined in tissues at 96 h following dosing.

<sup>14</sup>C-Thiram was well absorbed by both sexes (>83% of the dose) following oral administration. Radioactivity was excreted in the urine (35-40% of the dose within 96 h), faeces (2-5%), and expired air (41-48%). Excretion was more extensive and rapid in urine and expired air within the first 12 h post-dosing, while the majority of the faecal radioactivity was excreted after 24 h. The majority of the dose (at least 83.7 and 89.6% for male and female rats, respectively) was eliminated from the body within four days post-dosing. Sex did not affect the extent or rate of excretion of orally administered <sup>14</sup>C-thiram. Trace levels of radioactivity were detected in all tissues analyzed at 96 h after dosing. In general, the highest concentrations were in liver, blood cells and kidneys, and the lowest were in brain, plasma, and skeletal muscle. About 2-3% of the dose remained in tissues after four days. The total recovery of radioactivity averaged about 85 and 93% for males and females, respectively (Nomeir & Markham, 1990).

The bioavailability of radioactivity from a single feeding with a diet containing 30 ppm equal to 1.5 mg <sup>14</sup>C-thiram/kg bw (mixture of labelled (purity 92.4%) and non-labelled substance (purity 97.5%)) was evaluated in five male Charles River Crl:CD<sup>R</sup>BR albino rats (body-weight 246-270 g). The specific activity of <sup>14</sup>C-thiram was 15 mCi/mmol. The stability of the test materials was satisfactory. Test rats, conditioned to consume an entire day's feed during the last hour of their dark cycle, were fed once with <sup>14</sup>C-thiram-containing diet and then placed in metabolism cages. Radioactivity in the urine, faeces and expired air (carbon dioxide and carbon disulphide) over the next 72 h and the residues in the carcass and gastrointestinal tract at 72 hours were determined. The expired gases contained 41% of the administered radioactivity, urine contained 38% and carcass contained 6%. The faeces and gastrointestinal tract accounted for 20% of the dose. The total recovery of radioactivity averaged 105%.

It was demonstrated that 85% of the radioactivity equivalents was absorbed into the systemic circulation of the rat. Assumptions were made that there was no elimination of absorbed radioactivity into the gastrointestinal tract and that all of the radioactivity in the expired gases, urine, and carcass represented absorbed thiram (Hiles, 1989).

## Biotransformation

The metabolic pathway of thiram in rats is shown in Figure 1.

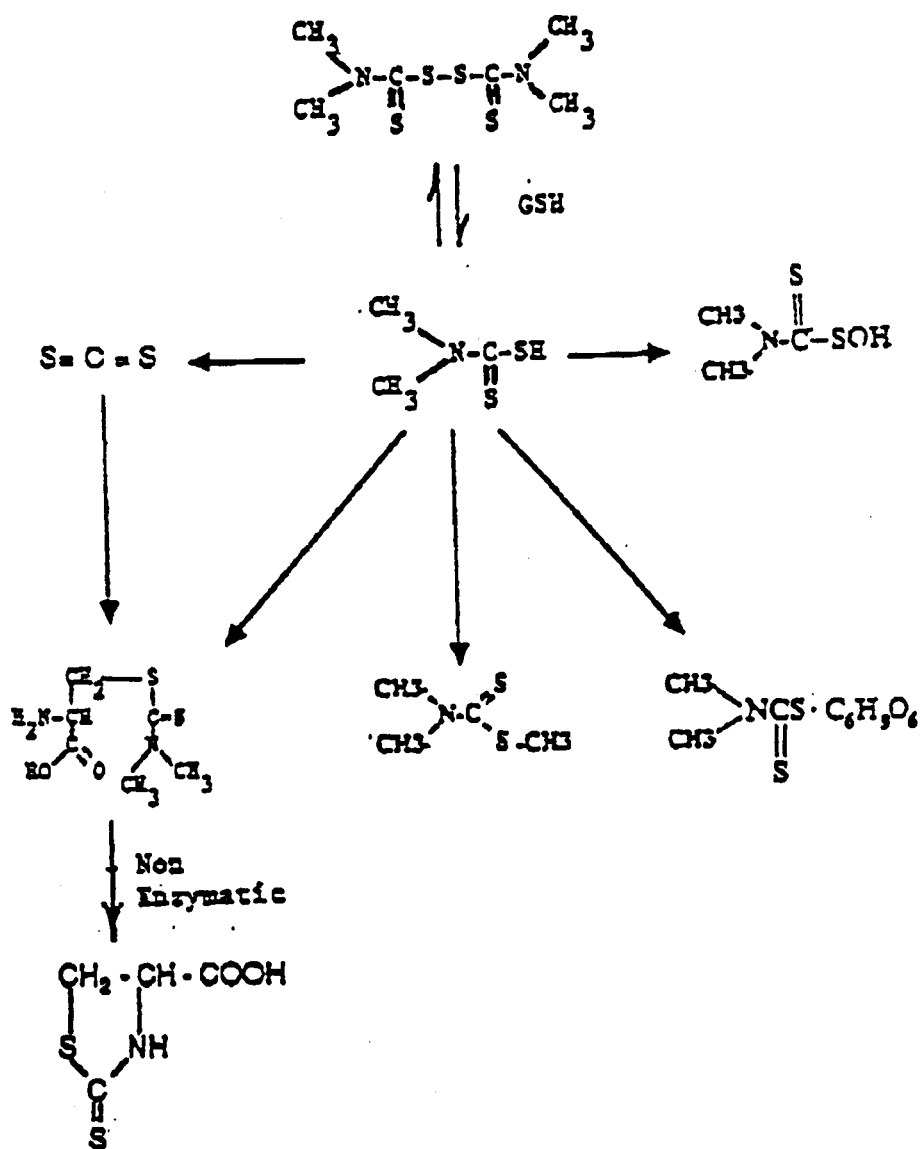
Male Sprague-Dawley rats (160-200 g from Southern Animal Farms) were injected i.p. with 15, 30 or 60 mg thiram (purity 99%)/kg bw (solvent corn oil). Following injection rats were placed individually in a 4-litre all-glass metabolic cage. The expired air was drawn, over a period of 5 h, through traps containing 1N NaOH (1 trap), then through ethanol-diethylamine-triethanolamine-cupric acetate reagent (2 traps). The amount of carbon disulphide dissolved in the reagent in the traps was determined colorimetrically using a spectrophotometer. In a similar experiment, 60 mg thiram/kg bw was given to rats previously treated intraperitoneally with 40 mg SKF 525-A/kg bw (1 dose 30 min earlier) or with 5 daily doses of 50 mg phenobarbital/kg bw. In subsequent experiments, rats were treated i.p. with 60 mg thiram/kg bw and sacrificed at 5 and 24 hour intervals. Activities of hepatic microsomal and serum enzymes were measured. The extent of thiram-induced liver injury was assessed by ASAT and SDH activity.

A dose-dependent excretion of carbon disulphide (completed within 5 h was demonstrated in the expired air (2.6, 26, and 120 nmol at doses of 15, 30, and 60 mg/kg bw, respectively). Expiration of carbon disulphide, was increased by phenobarbital pre-treatment and decreased by SKF 525-A pre-treatment. Activities of hepatic microsomal and serum enzymes indicated that thiram caused a significant loss of cytochrome P-450 and benzphetamine N-demethylase activity and significant elevation of SDH and ASAT activity. It was concluded that carbon disulphide is an *in vivo* metabolite of thiram and may be responsible for the observed hepatotoxicity (Dalvi & Deoras, 1986).

The volatile <sup>14</sup>C-residues in the expired air were determined in Charles River Sprague-Dawley rats (age 7 weeks). Following 10 days acclimatization, three rats received an oral dose of 2.1-2.5 mg <sup>14</sup>C-thiram/kg bw (purity 98%, specific activity 20.7 mCi/mmol). Each rat was then placed in a sealed glass metabolism cage for 96 hours. The exit gases from the metabolism cage were passed through a scrubbing tower containing Harvey Carbon-14 Cocktail (efficient at trapping CO<sub>2</sub>, CS<sub>2</sub>, and COS), then through a solution of diethylamine/ethanol and finally through a solution of Viles CS<sub>2</sub> reagent. Air flow through the metabolism cage was 3.5-4.0 l/min. Urine was also collected and radioassayed.

An average of 61% of the radioactive dose was recovered as volatiles. More than 99.5% of the volatiles was recovered in the Harvey Carbon-14 Cocktail. Labelled residues in urine accounted for 25-43% of the radioactive dose. The total radioactivity recovered in the volatiles and the urine for the three rats averaged 94% of the dose. Results indicate that thiram is largely metabolised into volatiles such as CO<sub>2</sub>, CS<sub>2</sub>, and COS. The study does not allow a characterization or quantification of the three metabolites (Norris, 1989).

Figure 1. The metabolic pathway of thiram in rats



The identification of thiram metabolites in urine was determined in 2 Charles River Crl:CD<sup>R</sup>(SD)BR rats/sex. The rats (approximately 5 weeks old) were fed diets containing 50 ppm unlabelled thiram for nine weeks followed by a single oral dose of <sup>14</sup>C-thiram (purity 99%, specific activity 15.5 mCi/mmol). Samples of urine were collected over the first 24 h after treatment termination and analyzed by HPLC.

Approximately 60% of the administered radioactivity was recovered as expired CS<sub>2</sub> and 30% was found in the urine. Thiram was rapidly degraded to more polar products. Virtually no unchanged thiram was detected in the urine. Five urinary metabolites were detected by HPLC and were identified by mass spectrometry. The identified metabolites were an alanine derivative of CS<sub>2</sub> (10%); a glucuronide conjugate of dimethyldithiocarbamate (DDC) (20%); a thiosulfenic acid (34%); the methyl ester of DDC (6%); and an alanine conjugate (30%). The presence of these polar conjugates demonstrates that the metabolic pathway involved a reduction of the disulphide bond and subsequent reactions of the thiol moiety to form oxidative and conjugative polar products (McManus, 1991).

### Toxicological studies

#### Acute toxicity studies

**Table 1. Acute toxicity of thiram**

Species	Sex	Route	LD <sub>50</sub> (mg/kg bw)	LC <sub>50</sub> (mg/m <sup>3</sup> )	References
Mouse	M	oral	4000		Matthiask, 1973
Mouse	F	oral	2300-3800		Lee <i>et al.</i> , 1975; 1978
Rat	M	oral	3700-4000		Thouin, 1985a; Lee <i>et al.</i> , 1975; 1978
Rat	F	oral	1800-1900		Thouin, 1985a; Lee <i>et al.</i> , 1975; 1978
Rat	M&F	inhalation		> 100	Debets, 1985
Rabbit	M&F	dermal	> 2000		Thouin, 1985b

#### Short-term toxicity studies

##### Mice

Ten Charles River albino mice (Crl:CD-1<sup>R</sup>(ICR)BR)/sex/group (males 24.3-24.8 g, females 19.0-19.3 g) received in the diet 0, 300, 600, or 1200 ppm thiram (purity 97.5%) for 4 weeks equal to 0, 54, 108 or 201 mg/kg bw/day in males and 62, 118 or 241 mg/kg bw/day in females. Stability and homogeneity of the test substance was satisfactory. The animals were observed daily for mortality, signs of

toxicity and food consumption. Body-weights were recorded weekly. Haematology and clinical chemistry tests were performed at sacrifice on days 29 or 30. Gross and histopathological examinations of the animals were carried out.

Body-weights were significantly and dose-dependently reduced in males at all dose levels. Food consumption was significantly lower for both sexes at all dose levels when compared with controls. Statistically significant differences in clinical chemistry and haematological parameters included reduced erythrocyte counts, haemoglobin, and haematocrit for males at all dose levels; increased platelet counts for females at 600 and 1200 ppm; reduced serum glucose for females at 1200 ppm. Statistically significant and dose-dependent increased organ-to-body-weight ratios of the brain, kidneys and liver in males probably resulted from statistically significant and dose-dependent reduced terminal body-weights. A NOAEL could not be determined because of the 28-48% reduction in food intake at 300 ppm (Kehoe, 1989b).

### Rats

In a 13-week study, 10 Charles River rats (Crl:CD<sup>R</sup>(SD)BR)/sex/group (males 138 ± 6 g and females 125 ± 4 g) received dietary concentrations of 0, 50, 500, or 1000 ppm thiram (purity 99.4%) equivalent to 0, 2.5, 25 or 50 mg/kg bw/day. Mean stability assay results for diets stored frozen for 14 and 35 days ranged from 81-99%. Diets analyzed after being in the animals room for 7 days were 17, 80, and 88%, respectively, of the nominal levels. Mean values for homogeneity assay results were 80, 97, and 98%, respectively, of the nominal levels of 50, 500, and 1000 ppm. The mean ± SD of determined concentration of thiram was 40 ± 1, 483 ± 8, and 976 ± 15 ppm. The animals were observed daily for mortality and signs of toxicity. Body-weights and food consumptions were recorded weekly and haematology and clinical chemistry were determined at study termination. Ophthalmic observations were done before and at termination of study. Gross and histopathological examinations from the control and 1000 ppm group and of macroscopic lesions, lungs, liver, and kidneys from all 50 ppm and all 500 ppm animals was carried out at study termination.

Body weights, cumulative body-weight gains, and food consumption were significantly reduced throughout the study for both sexes at 500 and 1000 ppm. Changes in clinical chemistry and haematological parameters occurred at dose levels of 500 and 1000 ppm. The changes considered to be treatment-related were reduced erythrocyte counts, haemoglobin and haematocrit in females; increased MCV and MCH in both sexes; increased white blood cell, corrected white blood cell, absolute neutrophil, absolute lymphocyte and absolute monocyte counts in females; reduced total protein and glucose in both sexes; reduced albumin and increased urea nitrogen and chloride in females.

At 500 and 1000 ppm animals a tendency to reduced terminal body-weights with correspondingly reduced absolute organ weights and increased organ to body-

weight ratios were observed. Macroscopically, the non-glandular stomach in some animals showed areas of erosion and the mesenteric lymph nodes were diffusely red or mottled. Microscopically, the mucosa of the nonglandular stomach had focal areas of erosion/ulceration, mucosal hyperplasia, or both, accompanied by some submucosal inflammation and edema. These changes appeared to be treatment-related. The mesenteric lymph nodes were frequently congested but otherwise normal. The NOAEL in this study was 50 ppm, equivalent to 2.5 mg/kg bw/day (Kehoe, 1988b).

## Dogs

Two acclimated beagle dogs (5 months of age and weighing 5.6 to 8.5 kg/sex/group) were fed dietary concentrations of 0, 125, 500, or 2000 ppm thiram (purity 99.4%), equivalent to 0, 3, 13 or 50 mg/kg bw/day for 4 weeks. All animals were observed daily for toxic signs and mortality. Body-weight and food consumption was recorded weekly. Clinical chemistry, gross and histopathological examinations were performed on all animals. Homogeneity assays indicated from 94-96% (mean values) of the nominal values. There were no data on stability, but thiram was stored under similar conditions to that in other studies where stability was satisfactory.

One 2000 ppm one female died during week 2 and one male became moribund and was sacrificed during week 4. Therefore, the high-dose of 2000 ppm was reduced to 1500 ppm during weeks 3 and 4. The observations in the female were primarily colon changes associated with the acute death and subsequent post-mortem change. The male had only a few incidental macroscopic and microscopic findings and, therefore, its death was not considered compound-related. At week 4, body-weights for both sexes at 500 ppm were approximately 15% lower and at 2000/1500 ppm, 33 and 39% lower than controls respectively. Food consumption during week 4 for 125, 500, and 2000/1500 ppm males were 12%, 29%, and 92% lower than controls respectively, and for females 13%, 49%, and 78% lower. Treatment-related changes in clinical chemistry and haematological parameters comprised reduced erythrocyte counts, haemoglobin and haematocrit in males given 500 or 2000/1500 ppm. One male given 2000/1500 ppm had increased ALAT, ASAT and ALP. Slightly reduced absolute lymphocyte counts and slightly higher platelet counts, total bilirubin and urea nitrogen occurred in some male and female dogs given 500 or 2000 ppm. The surviving 2000/1500 ppm dogs sacrificed at week 4 had notably reduced terminal body-weights and correspondingly reduced absolute organ weights. The liver of the males had hepatocellular degeneration with sinusoidal cell proliferation and pigmentation. There were no major macroscopic or microscopic findings in the remaining dogs. The NOAEL for the study was determined to be 125 ppm, equivalent to 3 mg/kg bw/day, based on the lower body-weights and changes in clinical chemistry parameters (Kehoe 1988a).

In a 13-week study on 5 month old beagle dogs, four beagle dogs/sex/group were fed dietary concentrations of 0, 75, 250, or 500 ppm thiram (purity 97.5%),

equal to 0, 2.2, 6.9 or 12 mg/kg bw/day in males and 0, 2.3, 7.3 or 13 mg/kg bw/day in females. The mean ( $\pm$  SD) body-weight at study initiation was  $7.4 \pm 0.6$ ,  $7.3 \pm 1.1$ ,  $7.4 \pm 0.5$ , and  $7.3 \pm 0.6$  kg for males receiving 0, 75, 250, and 500 ppm. and were  $6.8 \pm 0.9$ ,  $7.1 \pm 1.4$ ,  $6.3 \pm 0.5$ , and  $6.7 \pm 0.5$  kg for females. All animals were observed daily for clinical signs, moribundity and mortality. Body-weight and food consumption was recorded weekly. Ophthalmic examinations were performed prior to and at the end of the study. Clinical chemistry measurements, haematological examinations and gross and histopathology examinations were done on all animals. Homogeneity of diets was within  $\pm 8\%$  of nominal values. Mean stability assays of diets frozen for 7 days, stored in the animal room for 1 day and then frozen for 4 weeks, were within  $\pm 5\%$  of nominal levels.

Body weights for the 500 ppm dogs were significantly reduced. Food consumption was significantly reduced in 500 ppm males and in 250 and 500 ppm females. Treatment-related effects for clinical chemistry and haematological parameters were reduced erythrocyte counts, higher MCV and MCH in 75, 250, and 500 ppm dogs; reduced haemoglobin and haematocrit in 500 ppm females; higher platelet counts in 250 and 500 ppm males; lower total protein and albumin in 75, 250, and 500 ppm males and females; and higher cholesterol in 250 and 500 ppm males and females. The NOAEL for this study was 75 ppm equal to 2.2 and 2.3 mg/kg bw/day in males and females respectively, based on haematological changes noted in both sexes (Kehoe, 1989a).

Six 4 to 5 month old beagle dogs/sex/group (initial weight 4.0 to 6.8 kg) were fed dietary concentrations of 0, 30, 90, or 250 ppm thiram (purity 97.5%), equal to 0, 0.84, 2.6, and 7.4 mg/kg bw/day in males and 0, 0.90, 2.5, and 7.2 mg/kg bw/day in females for 52 weeks. All animals were observed daily for clinical signs and mortality. Body weights and food consumption were recorded weekly for weeks 1 through 16, and monthly thereafter. Ophthalmic examinations were performed prior to and at the end of the study. Haematology, clinical chemistry, gross and histopathology were performed on all animals. Mean values for homogeneity assays were 83, 92, and 92% of the nominal levels of 30, 90, and 250 ppm, respectively. Mean stability assay results for diets kept below 0 °C for 7 days and stored in the animal room for one day were 84%, 92% and 96% of the nominal levels of 30, 90, and 250 ppm, respectively.

No ophthalmic lesions were observed in any animals. Erythrocyte counts were reduced in males given 250 ppm, total protein was lower and cholesterol was higher in males given 90 or 250 ppm and in females given 250 ppm. Albumin was reduced in both sexes given 250 ppm. Absolute liver weights in males given 90 or 250 ppm were significantly increased. Liver-to-body-weight ratios in males given 30, 90, or 250 ppm and in females given 250 ppm were also significantly increased, as were liver-to-brain weight ratios in males given 250 ppm. The significant increase in liver-to-body-weight ratios in males given 30 ppm was due to a slight increase in absolute liver weight and a slight decrease in body weight. Furthermore, the observation that the body weights of males given 90 ppm were

increased in relation to those of the controls and those of males given 30 ppm demonstrates, that the slight decrease in body weights seen in the 30 ppm group was spurious. Thus, the increase in liver-to-body-weight ratio seen in males given 30 ppm was not considered a test material-related effect. The changes in liver weights, in conjunction with the altered total protein, albumin, and cholesterol levels suggest a test material-related effect on liver function and size. These changes were probably adaptive responses to the presence of this compound. Based on increased absolute liver weights and altered clinical chemistry parameters in males given 90 or 250 ppm, and altered clinical chemistry parameters in females at 250 ppm, the NOAEL for male dogs was 30 ppm, equal to 0.84 mg/kg bw/day, and the NOAEL for female dogs was 90 ppm equal to 2.5 mg/kg bw/day (Kehoe 1991a).

Four groups of 4 beagle dogs/sex/dose were treated orally via gelatin capsule with thiram (purity 98.7%) at doses of 0 (blank capsule), 0.4, 4, or 40 mg/kg bw/day for 104 weeks. Body weights and food intake were measured weekly. Water consumption, haematology, ophthalmoscopy, clinical chemistry and gross and histopathology of all organs were performed.

Both sexes at 40 mg/kg bw/day showed severe toxic signs including nausea, vomiting, salivation, and clonic convulsion and severe anaemia within the first 11 weeks of treatment and were all subjected to unscheduled necropsy before the 29th week of treatment. The dogs also showed ophthalmological changes such as fundal haemorrhage, miosis, and desquamation of the retina which were consistent with retinal lesions observed histopathologically. In the 4 mg/kg bw/day group, nausea, vomiting and salivation were common findings in both sexes and one female showed clonic convulsion from week 37. Parameters including haematocrit, haemoglobin, and erythrocyte count were depressed from week 4 in both sexes treated with 4 or 40 mg/kg bw/day, indicating anaemia. Changes in biochemical parameters indicating liver failure were observed from week 4 in the 40 mg/kg bw/day males and later in both sexes dosed with 4 or 40 mg/kg bw/day. Kidney damage was detected in the histopathological examination in two female dogs in each of the 4 and 40 mg/kg bw/day groups. Histological lesions in the central or peripheral nervous system relating to the observed neurological disturbances were not found. It was concluded that no sex difference in response to treatment was demonstrated. Based on the neurological disturbances, anaemia and the effects on the liver, the NOAEL was 0.4 mg/kg bw/day (Maita *et al.*, 1991).

### **Long-term toxicity/carcinogenicity studies**

#### **Mice**

Four groups of 50 acclimated (24 days) Charles River Crl:CD-1<sup>R</sup>(ICR)BR mice/sex/dose level (approximately 7 weeks of age) were fed thiram (97.5% purity) at dietary concentrations of 0, 15, 150, or 300 ppm (males), equal to 3, 24 or 50 mg/kg bw/day and 0, 15, 300, or 600 ppm (females), equal to 3, 57 or

112 mg/kg bw/day for 97 weeks, when the most sensitive group showed 50% mortality. Homogeneity assays indicated from 75-84%, 95-100%, 94-103%, and 101-103% of the nominal levels at 15, 150, 300, and 600 ppm, respectively. Mean stability assay results for diets frozen for 6 days and stored in the animal room for one day were 55, 90, 90, and 97% of the nominal levels at 15, 150, 300, and 600 ppm, respectively, demonstrating stability of varying acceptability. Survival, clinical signs, body weights, food consumption, feed efficiency, haematological parameters, necropsy, absolute and relative organ weight and histopathological examination were studied.

No substance-related oncogenic effects or adverse effects on survival were observed in any test group. No changes attributable to treatment were seen in any of the parameters evaluated in the 15 ppm groups. There was no clinical indication of neurotoxicity at any dose level. Mean body weight, weight gain, and total food consumption were statistically significantly decreased in a dose-dependent manner in the mid- and high-level groups beginning at weeks 4-5. At study termination, mean weights were 7%, 15%, 14%, and 19% below control and mean total food consumption was 6%, 10%, 12%, and 17% below control for the 150 ppm males, 300 ppm males, 300 ppm females, and 600 ppm females, respectively. Increased frequency of sores or reddened areas on the skin, generally on the ears, consistent with bacterial dermatitis was noted for the 300 ppm males and 600 ppm females. Mild but significant decreases in mean erythrocyte count, haemoglobin, and haematocrit values were seen for the 600 ppm females at week 97. Histopathology showed no evidence of thiram-induced neoplasia. Non-neoplastic findings in the mid- and high-level male and female mice consisted of retinal atrophy, intracytoplasmic protein-like droplets in the urinary bladder superficial transitional epithelium, and necrosis and suppurative inflammation in the skin of the 150 and 300 ppm males and 300 and 600 ppm females; hyperkeratosis in the nonglandular stomach of the 300 ppm males and 300 and 600 ppm females; and increased pigment in the spleen and decreased pigment in the inner adrenal cortex of the 300 and 600 ppm females.

No oncogenic effect was observed in doses up to 300 ppm, equal to 50 mg/kg bw/day in males and 600 ppm, equal to 112 mg/kg bw/day in females. Based on the body-weight reduction, the NOAEL for long-term toxicity was 15 ppm, equal to 3 mg/kg bw/day (Trutter, 1992).

#### Rats

Four groups of 64 Jcl:Wistar rats were fed dietary concentrations of 0, 3, 30, or 300 ppm thiram (purity 98.7%), equal to 0, 0.1, 1.2 or 12 mg/kg bw/day for males and 0, 0.1, 1.4 or 14 mg/kg bw/day for females, for 104 weeks. Body weights, food intake, and water intake were measured weekly up to week 52 and monthly thereafter. Haematology, clinical chemistry, ophthalmoscopy and gross and histopathology were performed on all rats. Test diets were prepared twice weekly

during the study. Data on homogeneity were not available, but diets were stable up to 1 week.

Mortality of females in the 30 and 300 ppm groups was slightly increased compared to controls during the last 8 weeks of treatment, apparently correlated to an incidental higher occurrence of pituitary tumours during this period. Overall incidence of pituitary tumours in females was comparable to that in the control group. Decreased body-weight gain and reduced food intake were observed in both sexes at 300 ppm. Anaemia and regressive changes in the sciatic nerve accompanied by atrophy of the calf muscle (*M. triceps surae*) were seen in the 300 ppm females. During the last 8 weeks of the treatment period, the incidence of pituitary adenomas was increased in females of the 30 and 300 ppm groups compared to that in controls. Because of the small number of animals, statistical analysis was not carried out. Statistical analysis of pituitary tumour incidence in all animals at the end of the experiment showed no significant differences. No evidence of carcinogenic potential was observed. Based on mortality, anaemia, nerve degeneration and muscle atrophy a NOAEL of 30 ppm, equal to 1.2 and 1.4 mg/kg bw/day was determined in males and females, respectively (Miata *et al.*, 1991).

Four groups of sixty 36-day old Charles River Crl:CD<sup>R</sup>(SD)BR rats/sex/group (initial weights 125-161 g for males and 115-193 g for females) were fed dietary concentrations of 0, 30, 150, or 300 ppm thiram (purity 97.5%), equal to 0, 1.5, 7.3 or 15 mg/kg bw/day in males and 0, 1.8, 8.9 or 19 mg/kg bw/day in females, for 104 weeks. Mean values for stability assays of diets kept below 0 °C for 6 days, animal room for one day, then below 0°C for 30 days were 15.5 ppm (52%), 123 ppm (82%), and 260 ppm (87%) for nominal contents of 30, 150, and 300 ppm, respectively. The values for homogeneity assay results ranged from 21.8-24.2 (73-81%), 128-138 (85-92%), and 255-276 ppm (85-92%) for nominal contents of 30, 150, and 300 ppm, respectively. Cumulative means for the routine diet analysis for dose confirmation were 16.3 (54%), 119(79%), and 262 ppm (87%) and the assay results ranged from 4.41-27.5 ppm, 81.8-151 ppm, and 179-320 ppm of the nominal dose levels of 30, 150, and 300 ppm, respectively, through 104 weeks. Data from a parallel study with <sup>14</sup>C-thiram stored under similar conditions (Hiles, 1989) showed, that animals on a diet containing 30 ppm could be expected to receive a systemic exposure to thiram and/or thiram-derived materials approximately equal to the amount added to the diet, even though the expected level could not be verified analytically. Therefore, these data were considered to demonstrate that the anticipated levels of thiram were achieved in the test diets. The animals were observed daily for clinical signs and mortality. Body-weight and food consumption were recorded weekly and haematology, clinical chemistry, and urine analysis parameters were evaluated during the study and at study termination. Ophthalmic observations were done before and at termination of the study and gross and histopathology examinations were carried out at study termination.

Clinical signs possibly test material-related for males included swollen nose, soft faeces and opaque eyes. Soft faeces were possibly test material-related at 150 and 300 ppm for the females. Body-weight and cumulative body-weight gains in both sexes were statistically significantly lower than those of the controls at 150 and 300 ppm. Food consumption was statistically significantly lower than that in the controls for both sexes given 30, 150, or 300 ppm. Reduced erythrocyte counts, haemoglobin, haematocrit and higher mean corpuscular volume and mean corpuscular haemoglobin were observed in females given 150 or 300 ppm. Statistically significant positive trend analyses for hepatocellular adenomas (both sexes) and thyroid C-cell adenomas (terminal sacrifice males and terminal plus unscheduled sacrificed females) were reported but individual group comparisons with controls were statistically insignificant. There was no increase in the incidence of thyroid C-cell carcinomas. Extramedullary haematopoiesis in the liver appeared to be increased in males at 150 or 300 ppm and in females at 300 ppm. The biological significance of this finding is unclear. Steatosis/fatty infiltration of the pancreas (not uncommon in old SD rats) appeared to be increased for males and females given 150 or 300 ppm. Also in the pancreas, multifocal acinar atrophy was more common for males given 150 or 300 ppm. The incidence of bile duct hyperplasia showed a statistically significant positive trend in females, but was statistically significantly increased only at 300 ppm. There were no histopathological findings recorded that suggested test material-related neurotoxicity.

Based on statistically significant decreases in body weights and cumulative body-weight gains for males and females given 150 or 300 ppm, effects on haematological parameters in females given 150 or 300 ppm, and histopathological changes in males and females given 150 or 300 ppm, the NOAEL was 30 ppm, equal to 1.5 and 1.8 mg/kg bw/day in males and females, respectively. Thiram was not found to be carcinogenic at doses up to 15 and 19 mg/kg bw/day in males and females, respectively (Kehoe, 1991b).

Three groups of 50 five-week old Charles River SPF F344 rats, initial mean body-weight of about 200 g for males and about 150 g for females, were fed dietary concentrations of 0, 500 or 1000 ppm, equal to 0, 18 or 39 mg/kg bw/day in males and 0, 20 or 42 mg/kg bw/day in females for 104 weeks. Body weights and food consumption were measured at regular intervals and haematology and clinical chemistry were evaluated during the study and at study termination. Gross and histopathology were performed. Homogeneity data and stability data were not given.

Reduced body-weight gain and food consumption were seen at 500 and 1000 ppm in both sexes but especially in females of the high-dose group. In males reduced liver function was observed. Mononuclear cell leukaemia was found with an incidence of 20%, 8%, and 4% in males and 29%, 12%, and 4% in females for 0, 500, and 1000 ppm animals, respectively. No tumour induction related to the treatment was observed. It was concluded that thiram had no carcinogenic effect

at doses up to 39 and 42 mg/kg bw/day in males and females, respectively (Hasegawa *et al.*, 1988).

### Reproduction studies

In a two-generation reproduction study, thiram (purity 97.6%) was administered to Charles River Crl:CD VAF/Plus rats in dietary concentrations of 0, 30, 60 or 180 ppm equal to 0, 1.5, 2.9 or 8.9 mg/kg bw/day in the F<sub>0</sub> males and 0, 2.3, 4.6 or 14 mg/kg bw/day in F<sub>0</sub> females, and 0, 1.8, 3.8 or 11 mg/kg bw/day in the F<sub>1</sub> males and 0, 2.4, 5.1 or 16 mg/kg bw/day in the F<sub>1</sub> females. Homogeneity of thiram was satisfactory in the mixed diets. The test compound was stable kept frozen for 7 days and reasonably stable (losses of 18%, 16%, and 9% in the 30, 60, and 180 ppm diet, respectively) kept at room temperature for 12 hours. The mean  $\pm$  SD percent of target ppm found in all analyzed and administered 30, 60, and 180 ppm diets was  $93 \pm 6.3$ ,  $94 \pm 6.6$ , and  $98 \pm 5.2\%$ , respectively. The F<sub>0</sub> and F<sub>1</sub> parental generation consisted of 26 males and 26 females per group. F<sub>0</sub> animals were treated beginning at 63 days of age for 81 days prior to the initial mating (F<sub>1a</sub>). A second litter (F<sub>1b</sub>) was produced after a rest period of 16 days following weaning of the F<sub>1a</sub> litter. Since conception rates were poor across all groups, including the controls, in the F<sub>1b</sub> litter, an F<sub>1c</sub> litter was also produced. The F<sub>1</sub> parents were selected from the F<sub>1c</sub> litters. These animals were treated beginning at 22 days of age for a minimum of 84 days prior to their initial (F<sub>2a</sub>) mating. The F<sub>1</sub> parents were mated twice to produce the F<sub>2a</sub> and F<sub>2b</sub> litters. All parental animals, and pups were observed daily for mortality and overt toxicity. Detailed clinical observations were recorded at least once a week. Reproductive and litter parameters assessed included male and female fertility indices, events at parturition, gestation length, litter size, numbers of viable and stillborn pups, and offspring survival and growth during lactation. All parental animals, and all F<sub>1c</sub> weanlings not selected to remain on study and all F<sub>2b</sub> weanlings were subjected to a gross necropsy. The F<sub>1a</sub>, F<sub>1b</sub>, and the F<sub>2a</sub> offspring were euthanized and discarded following weaning.

Mean maternal body weights and mean food consumption of the F<sub>0</sub> females were reduced at 60 and 180 ppm during the F<sub>1a</sub> gestation period. Reductions in mean maternal body-weight and/or food consumption were also observed at 180 ppm during the F<sub>1b</sub> and F<sub>1c</sub> gestation periods and the F<sub>1a</sub>, F<sub>1b</sub>, and F<sub>1c</sub> lactation periods. Similar reductions were observed for the F<sub>1</sub> females at 180 ppm during the F<sub>2a</sub> and F<sub>2b</sub> gestation and lactation periods. Reductions in mean weekly food consumption of the F<sub>0</sub> males and females were observed at 60 and 180 ppm. Mean offspring body weights were consistently reduced to a significant degree in all litters across both generations at the 180 ppm level. Apart from a consistent statistically significant reduction in body weights in the top dosed offspring during the F<sub>1b</sub> and F<sub>1c</sub> gestation periods and the F<sub>1a</sub>, F<sub>1b</sub>, and F<sub>1c</sub> lactation periods, no substance-related changes were found in the reproductive parameters or in the parameters for post-natal development.

Based on the findings of parenteral systemic toxicity, the NOAEL was 30 ppm, equal to 1.5 and 2.3 mg/kg bw/day in males and females, respectively. With respect to filial systemic toxicity, the NOAEL was 60 ppm, equal to 3.8 and 5.1 mg/kg bw/day in males and females, respectively. With respect to reproduction and post-natal development, the NOAEL was greater than 180 ppm equal to 8.9 and 14 mg/kg bw/day in males and females, respectively (York, 1991).

### Special studies on genotoxicity

Data are shown in Table 2.

### Special studies on teratogenicity

In a preliminary oral teratology study, groups of 6 Charles River CD strain (Sprague-Dawley origin) rats (196-229 g) were administered dose levels of 0, 5, 10, 20, 40, or 80 mg/kg bw/day (volume-dosage 10 ml/kg bw) by gavage from day 6 through day 15 of gestation. Control rats received the vehicle, 0.5% (W/V) CMC and 0.5% (W/V) Tween 80 in distilled water. Homogeneity and stability of thiram in the suspensions were satisfactory (mean concentrations  $\pm$  SD of the 0.5, 1.0, 2.0, 4.0, 8.0, and 16 mg/ml thiram suspensions were  $0.5 \pm 0.004$ ,  $0.97 \pm 0.003$ ,  $1.96 \pm 0.05$ ,  $3.94 \pm 0.12$ , and  $8.06 \pm 0.25$ , and  $16.6 \pm 0.2$  mg/ml, respectively). All females were killed on day 20 of gestation for examination of uterine contents.

A dose-related loss of body weight was seen at 20, 40, and 80 mg/kg bw/day. Treatment at 40 and 80 mg/kg bw/day produced a dose-related increase in the total number of early and late resorptions and a consequent increase in post-implantation loss and reduction in viable litter size. It was concluded that the highest dose to be used in a main teratology study in the rat should not exceed 40 mg/kg bw/day (Tesh *et al.* 1986).

Four groups of 25 female Charles River CD SD rats (193-236 g, 9-11 weeks old) were administered dose levels of 0 (vehicle), 7.5, 15 or 30 mg (99.0-99.8% purity) thiram/kg bw/day by gavage on day 6 through 15 of gestation. Pre-pairing acclimatization was for 5 days. Test solutions (dosage volume, 10 ml/kg bw) were prepared in a 0.5% w/v CMC and 0.5% w/v Tween 80 vehicle. All females were killed on day 20 of gestation for examination of their uterine contents. Analyses showed that the concentrations of test substance in the test mixtures were 0.62-0.75 mg/ml, 1.47-1.49 mg/ml, and 2.88-2.91 mg/ml of intended concentrations of 0.75, 1.5, and 3.0 mg/ml, respectively. The test substance was stable at room temperature throughout the study. The concentration of tetramethylthiuram monosulphide was less than 0.01%.

Table 2. Results of genotoxicity assays on thiram

Test system	Test object	Concentration of thiram	Purity	Results	Reference
<i>In vitro</i>					
Ames test ( $\pm$ S9)	<i>S. typhimurium</i> TA 1535} TA 100} TA 1537} TA 1538}	1-100 $\mu$ g/plate (-S9) 1-1000 $\mu$ g/plate (+S9)	98.7%	+( $\pm$ S9) +( $\pm$ S9) -( $\pm$ S9) -( $\pm$ S9)	Poth (1990)
	TA 98	{10-1000 $\mu$ g/plate (-S9) {1-1000 $\mu$ g/plate (+S9)		-( $\pm$ S9)	
Gene mutation ( $\pm$ S9)	V79 Chinese hamster cells (HPRT-locus)	1-10 $\mu$ g/ml (-S9) 10-56 $\mu$ g/ml (+S9)	100%	-( $\pm$ S9)	Debets & Enninga (1986)
Chromosome aberrations ( $\pm$ S9)	Chinese hamster ovary cells	0.003-0.023 $\mu$ g/ml (-S9) 0.2-1.5 $\mu$ g/ml (+S9)	99.8%	-( $\pm$ S9)	Putman (1987a)
DNA repair (test)	Primary rat hepatocytes	0.03-10 $\mu$ g/ml	100%	- -	Weterings (1985)
SCE induction (with and without S-9 mix)	human lymphocytes	5-25 $\mu$ g/ml	98.6%	+	Perocco <i>et al.</i> (1989)

Table 2 (cont'd)

Test system	Test object	Concentration of thiram	Purity	Results	Reference
<i>In vivo</i>					
Mouse spot test	mouse (NMRI)	75, 750 mg/kg bw per os	98.7%	-	Völkner (1991)
Micronucleus test	mouse (CD-1)	38-377 mg/kg bw (i.p.)	99.8%	-	Putman (1987b)
Germ cell cyto- genetic assay	mouse (NMRI)	75-750 mg/kg bw per os	99.7%	-	Völkner (1990)
Germ cell cyto- genetic assay	mouse (Swiss albino)	total doses 80-320 mg/kg (gavage)	n.g.	(+)	Prasad <i>et al.</i> (1987)

n.g.:not given

With the exception of a dosage-related incidence in the number of females showing areas of hair loss on head, neck, back, and/or limbs, the general condition of females was comparable in all groups. At 15 and 30 mg/kg bw/day a transient, dose-related, loss of body-weight was observed. At 7.5 mg/kg bw/day the rate of weight gain was slightly, but significantly, reduced during the treatment period, but subsequent weight gain was similar to that of the controls. There were no adverse effects upon implantation or upon fetal survival, but fetal and placental weights were significantly reduced at 30 mg/kg bw/day. Placental weights were also slightly, but significantly, reduced in the 7.5 and 15 mg/kg bw/day animals, and foetal weight was slightly reduced at 15 mg/kg bw/day, but these values were within historical control ranges. At 30 mg/kg bw/day there was evidence of fetal immaturity e.g., reduced skeletal ossification and increased incidence of space between the body wall and organs, and there was a slightly increased incidence of subcutaneous oedema. At 15 and 30 mg/kg bw/day the incidence of 13th ribs of reduced size was slightly increased, but was not related to dosage. Three foetuses with diaphragmatic hernia were observed, two at 7.5 and one at 30 mg/kg bw/day. The NOAEL for fetal toxicity was 7.5 mg/kg bw/day. Reduced maternal body weight and placental weight precluded the establishment of a NOAEL for maternal toxicity. The toxic effects (immaturity and increased incidence of 13th ribs of reduced size) at higher doses were considered a result of maternal toxicity (Tesh *et al.*, 1988a).

### Rabbits

In a preliminary oral teratology study female New Zealand white rabbits (18-24 months old 3.4-4.5 kg) were artificially inseminated. The day of insemination was designated day 0 of gestation. The animals were allowed a minimum of three weeks acclimatization before dosing. Thiram (purity 99.1%) was administered by gavage to 4 females per group from days 6-19, inclusive, of gestation at dosages of 0 (vehicle), 1, 3, 5, 7.5, 10, 20, 40, or 80 mg/kg bw/day (volume-dosage 5 ml/kg bw). Control animals received the vehicle (the vehicle was 0.5% w/v aqueous CMC mucilage containing 0.5% w/v Tween 80). Stability and concentration (89-106% of nominal) of the test compound at room temperature was ensured by formulation of fresh test solutions each day. On day 29 of gestation, the animals were killed and their uterine contents examined.

Females receiving 10, 20, 40, or 80 mg/kg bw/day exhibited marked body-weight loss during the treatment period. Eight females (one on 20 mg/kg bw/day, three on 40 mg/kg bw/day and all four on 80 mg/kg bw/day) died or were killed in extremis. A further two deaths (one control and one on 40 mg/kg bw/day) occurred but were not attributed to treatment. Females receiving doses between 1 and 7.5 mg/kg bw/day showed slight reductions in their rate of body-weight gains with some indication of a dosage relationship. Treatment at 20 mg/kg bw/day was associated with total litter resorption in 2 females and a marked increase in post-implantation loss in the one female that carried a live litter to term. All 10 mg/kg bw/day females carried their litters to term, but there was a slight increase in post-

implantation loss compared with the controls. At 7.5 mg/kg bw/day one female showed total litter loss, but in the surviving two females post-implantation loss was similar to that of the controls. It was concluded that dose-levels of thiram for use in a main teratology study in the rabbit should not exceed 5 mg/kg bw/day to prevent effect on survival *in utero* (Tesh *et al.*, 1987).

New Zealand white rabbits 16-24 weeks of age and 3.7-4.8 kg body-weight, were artificially inseminated. The day of insemination was designated day 0 of gestation. The animals were allowed a minimum of three weeks acclimatization before dosing. Thiram (purity 99.5%) was administered by gavage from day 6-19 of gestation inclusive, at doses of 0, 1.0, 2.5 or 5.0 mg/kg bw/day (volume-dosage 5.0 ml/kg bw). The vehicle was 0.5% (W/V) aqueous CMC mucilage containing 0.5% (W/V) Tween 80. Stability and concentration of the test compound at room temperature were satisfactory. The concentration of tetramethylthiuram monosulphide was less than 0.03%. The number of control animals was 18+14 (initial controls + additional controls from a parallel study due to unusually poor pattern of body-weight gain, in part, to the performance of two females). The number of test animals per group were 15, 18, and 20 for the 1.0, 2.5, and 5.0 mg/kg bw/day dose group, respectively. All females were killed on Day 29 of gestation for examination. After removal of the reproductive tract the following was recorded: number of corpora lutea per ovary; number of implantation sites, number of resorption sites; number and distribution of live and dead fetuses in each uterine horn; weights and abnormalities of individual fetuses and placentae, thorough examination including skeletal examination of all fetuses.

The general condition of control and treated females was essentially similar throughout the investigation. Eight females died or were killed in extremis during the study (one in each of groups 1 and 2, two in group 3, and four in group 4). Necropsy findings revealed evidence of respiratory or gastrointestinal tract disorder, or accidental tracheal intubation. No evidence was apparent of any involvement of thiram. The body-weight gain of females receiving 5.0 mg/kg bw/day was slightly but statistically significantly reduced. Females receiving 1.0 or 2.5 mg/kg bw/day remained unaffected by treatment with thiram. One control female and one 5.0 mg/kg bw/day female aborted during the study. In addition, one female in each of the groups receiving 2.5 or 5.0 mg/kg bw/day exhibited total litter resorption. Litter parameters were unaffected by treatment with thiram. Survival, growth and morphological development *in utero* were essentially unaffected by treatment. The NOAEL for maternal toxicity was 2.5 mg/kg bw/day and the NOAEL for embryofetal toxicity was 5.0 mg/kg bw/day. There was no evidence of teratogenic potential (Tesh *et al.*, 1988b).

Five-month old New Zealand white SPF female rabbits were acclimatized and artificially inseminated. Groups of 20 inseminated rabbits were dosed once daily by oral gavage with 0, 1.0, 5.0, or 10 mg/kg bw/day (dose volume 3.0 ml/kg bw) thiram solved in 0.5% Tween 80 and 0.5% low-viscosity CMC on gestation days 7 through 19. Homogeneity mean values ranged from 107-108% of target and

the relative standard deviations were < 4.5%. Stability at room temperature for 10 days indicated 95-99% of the initial prepared concentrations. Caesarean sections were performed on all surviving females on gestation day 29, followed by teratologic examination of the fetuses. Individual fetuses were weighed, sexed, tagged and examined for external malformations and variations. Each fetus was dissected internally, sexed and examined for visceral malformations and variations. The head was examined using multiple coronal slices. Skeletal examination was carried out after Alizarin Red S staining.

A single death observed in the 10 mg/kg bw/day group was attributed to gavage injury from dosing and two deaths in the control group were attributed to pneumonia. No treatment-related adverse findings were observed with regard to clinical signs or necropsy findings of animals surviving the scheduled euthanasia. Statistically significant increases in body-weight gain and food consumption were observed in the treated groups when compared with the control values. These increases were not considered to be adverse effects of the test article. No treatment-related maternal effects were observed at cesarean section examination, and no adverse effects on fetal development were observed at the teratological examination. The NOAELs for maternal toxicity, embryofetal toxicity and teratogenicity were higher than 10 mg/kg bw/day (York, 1992).

#### **Special studies for neurotoxicity**

Data from special neurotoxicity testing of thiram were not available. Some endpoints related to neurotoxicity have been investigated in the standard toxicity testing procedures reported above.

#### **Observations in humans**

Workers, surgeons and other personnel using rubber gloves might get contact dermatitis due to the presence of thiram in the rubber (Lisi *et al.*, 1987). Oral exposure to thiram might lead to dermal sensitization to the substance (Goitre *et al.*, 1981).

#### **COMMENTS**

Following oral administration to rats, thiram was well absorbed (>83%) and eliminated via the expired air (41-48%), urine (25-40%), and faeces (2-5%). About 3% was recovered in various organs. The majority of the dose (84-90%) was eliminated within four days after dosing.

The metabolism of thiram was studied in rats. During the first five hours after administration, a dose-dependent formation of carbon disulphide was demonstrated in the expired air. Metabolites detected in urine included polar oxidation products and conjugates.

The acute oral toxicity of thiram is low in mice and rats. The World Health Organization has classified thiram as slightly hazardous (WHO, 1992).

A 13-week dietary study in rats at levels of 0, 50, 500 or 1000 ppm resulted in changes in haematological and serum biochemical parameters and gastric irritation at 500 and 1000 ppm. The NOAEL was 50 ppm, equivalent to 2.5 mg/kg bw/day.

Beagle dogs received thiram as a dietary admixture at levels of 0, 75, 250 or 500 ppm for 13 weeks or at levels of 0, 30, 90 or 250 ppm for 52 weeks. The NOAELs were 75 ppm (equal to 2.2 and 2.3 mg/kg bw/day in males and females, respectively, for the 13-week study) and 30 ppm (equal to 0.84 mg/kg bw/day) in males and 90 ppm (equal to 2.5 mg/kg bw/day) in females in the 52-week study on the basis of changes in body weight, increased absolute and relative liver weights, and changes in haematological and serum biochemical parameters. In another study, dogs received thiram in gelatin capsules at doses of 0, 0.4, 4 or 40 mg/kg bw/day 7 days/week for 104 weeks. Nausea, vomiting and salivation, ophthalmological effects, convulsions, changes in haematological parameters, and renal changes were observed at 4 and 40 mg/kg bw/day. On the basis of the described effects, the NOAEL was 0.4 mg/kg bw/day. Since thiram was administered in capsules in this experiment and significantly less information was available on the study conditions compared to those in the former two experiments with dietary administrations of thiram this NOAEL was not used as the basis for the estimation of an ADI.

In a 97-week oncogenicity study in mice using dietary thiram concentrations of 0, 15, 150 or 300/600 ppm, the effects included dose-dependent decreases of food consumption and body weight gain and changes in haematological parameters. Non-neoplastic findings included retinal atrophy, changes in the urinary bladder and in the skin, hyperkeratosis in the non-glandular stomach, and increased pigmentation in the spleen. Thiram was not carcinogenic in mice. The NOAEL for long-term toxicity in male and female mice was 15 ppm, equal to 3 mg/kg bw/day.

In a two-year toxicity study in rats at dietary concentrations of 0, 3, 30 or 300 ppm, the NOAEL was 30 ppm, equal to 1.2 and 1.4 mg/kg bw/day in males and females, respectively. It was based on lower red blood cell count, haemoglobin and haematocrit levels and degenerative changes of the sciatic nerve accompanied by atrophy of the gastrocnemius muscle at 300 ppm.

In a second two-year long-term toxicity/carcinogenicity study in rats at dietary concentrations of 0, 30, 150 or 300 ppm, dose-dependent lower erythrocyte counts, haemoglobin and haematocrit levels were observed. Based on these haematological changes, the NOAEL was 30 ppm, equal to 1.5 and 1.8 mg/kg bw/day in males and females, respectively.

In a 2-year carcinogenicity study in rats at dietary concentrations of 0, 500, or 1000 ppm (equal to 39 and 42 mg/kg bw/day in males and females, respectively) there was no evidence of carcinogenicity. The Meeting concluded that thiram was not carcinogenic in rats.

In a two-generation reproduction study in rats at dietary concentrations of 0, 30, 60 or 180 ppm, no adverse effects on reproduction were observed. The NOAEL for reproductive effects was > 180 ppm (equal to > 8.9 and > 14 mg/kg bw/day in males and females, respectively). The NOAEL for systemic toxicity was 30 ppm (equal to 1.5 and 2.3 mg/kg bw/day in males and females, respectively). This NOAEL was based upon reduction in body-weight and/or food consumption in both parental and offspring animals.

An oral teratogenicity study was performed in rats at gavage dose levels of 0, 7.5, 15 or 30 mg/kg bw/day. A NOAEL for maternal toxicity was not determined due to a dose-dependent decrease in body weight gain and placental weight at all dose levels. Teratogenicity was not observed.

In an oral teratogenicity study in rabbits at gavage doses of 0, 1.0, 2.5 or 5.0 mg/kg bw/day, a NOAEL of 2.5 mg/kg bw/day for maternal toxicity was based on a dose-dependent reduction of body-weight gain. Teratogenicity was not observed.

An oral teratogenicity study was carried out in rabbits at gavage dose levels of 0, 1.0, 5.0 or 10 mg/kg bw/day. The NOAEL for maternal toxicity was higher than 10 mg/kg bw/day. Teratogenicity was not observed.

Thiram was mutagenic in the Ames test but not in mammalian cells *in vitro*. Since thiram was not mutagenic *in vivo*, the Meeting concluded that it did not present a genotoxic hazard for humans.

The central and peripheral nervous systems have been recognized as a possible target for thiram toxicity. The neurotoxicity may be related to the thiram metabolite carbon disulphide.

An ADI was allocated, based on the 1-year study in dogs and the 2-year studies in rats, using a 100-fold safety factor.

## TOXICOLOGICAL EVALUATION

### Level causing no toxicological effect

Mouse:	15 ppm, equal to 3 mg/kg bw/day (97-week study)
Rat:	30 ppm, equal to 1.2 mg/kg bw/day (two-year study)
	30 ppm, equal to 1.5 mg/kg bw/day (two-generation reproduction study)
Rabbit:	2.5 mg/kg bw/day (teratology study, maternal toxicity)
Dog:	30 ppm in the diet, equal to 0.84 mg/kg bw/day (one-year study)

### Estimate of acceptable daily intake for humans

0-0.01 mg/kg bw.

### Studies which will provide information valuable in the continued evaluation of the compound

1. Clarification of the potential for neurotoxicity of thiram.
2. Observations in humans.

## REFERENCES

- Beauchamp, R.O., Bus, J.S., Popp, J.A., Boreiko, C.J. & Golberg, L. (1983) A critical review of the literature on the carbon disulphide toxicology. *CRD Crit. Rev. Toxicol.*, **11**: 169-272.
- Dalvi R.R. (1987) Dose-dependent liver toxicity of thiram administered intraperitoneally to rats. *J. Environ. Biol.*, **8**: 25-31.
- Dalvi, R.R. & Deoras, D.P. (1986) Metabolism of a dithiocarbamate fungicide thiram to carbon disulphide in the rat and its hepatotoxic implications. *Acta Pharmacol. Toxicol.*, **58**: 38-42.
- Debets, F.M.H. (1985) Evaluation of the acute inhalation toxicity of TMTD technical in the rat. Unpublished report no.: 0113/232 from NOTOX v.o.f. s-Hertogenbosch, the Netherlands. Submitted to WHO by UCB.

Debets, F.M.H. & Enninga, I.C. (1986) Evaluation of the mutagenic activity of TMTD technical in an *in vitro* mammalian cell gene mutation test with V79 Chinese hamster cells. Unpublished report No. NOTOX 0174/EV 1 from NOTOX C.V. DD 's-Hertogenbosch, Netherlands. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Gay, M.H. (1987) Rat metabolism of  $^{14}\text{C}$  thiram<sup>TM</sup>, single dose study. Unpublished report No. 87003B from Biotek, Inc., Massachusetts, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Goitre, M., Bedollo, P.G., & Cane, D. (1981) Allergic dermatitis and oral challenge to tetramethylthiuram disulphide. *Contact Derm.*, 7: 272-273.

Hasegawa, R., Takahashi, M., Furukawa, F., Toyoda, K., Sato, H., Junejang, J. & Hayashi, Y. (1988) Carcinogenicity study of tetramethylthiuram disulphide (thiram) in F334 rats. *Toxicology*, 51: 155-165.

Hiles, R.A. (1989) Bioavailability study in male rats with a  $^{14}\text{C}$ -thiram-treated diet. Unpublished report No. HLA 6111-131 from Hazleton Laboratories, Inc., Wisconsin, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Kehoe, D.F. (1988a) Four-Week range-finding study with thiram in dogs. Unpublished report No. HLA 6111-109 from Hazleton Laboratories, Inc., Wisconsin, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Kehoe, D.F. (1988b) Thirteen-Week toxicity study with thiram in rats. Unpublished report No. HLA 6111-110 from Hazleton Laboratories, Inc., Wisconsin, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Kehoe, D.F. (1989a) 13-Week toxicity study with thiram in dogs. Unpublished report No. HLA 6111-121 from Hazleton Laboratories, Inc., Wisconsin, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Kehoe, D.F. (1989b) 4-Week dietary range-finding study with thiram in mice. Unpublished report No. HLA 6111-127 from Hazleton Laboratories, Inc., Wisconsin, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Kehoe, D.F. (1991a) 52-Week dietary chronic toxicity study with thiram in dogs. Unpublished report No. HLA 6111-112 from Hazleton Laboratories, Inc., Wisconsin, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Kehoe, D.F. (1991b) 104-Week combined chronic toxicity and carcinogenicity study with thiram in rats. Unpublished report No. HLA 6111-113 from Hazleton Laboratories, Inc., Wisconsin, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Lee, C.C. *et al.* (1975) Toxicological evaluation of ferric dimethyldithiocarbamate (ferbam) and dithiocarbamate (thiram) with acute toxicity of manganese and zinc ethylenebisdithiocarbamates (maneb and zinc). Final report, MRI project no.: 36123-B. Submitted to WHO by UCB.

Lee, C-C. & Peters, P.J. (1976) Neurotoxicity and behavioral effects of thiram in rats. *Environmental Health Perspectives*, **17**: 35-43.

Lee, C.C. *et al.* (1978) Oral toxicity of ferric dimethyldithiocarbamate (ferbam) and tetramedthylthiuram disulfide (thiram) in rodents. *J. toxicol. environ. health*, **4**, 93-106. Submitted to WHO by UCB.

Lisi, P., Caraffini, S. & Assalve, D. (1987) Irritation and sensitization potential of pesticides. *Contact Derm.*, **17**: 212-218.

Maita, K., Tsuda, S. & Shirasu, Y. (1991) Chronic toxicity studies with thiram in wistar rats and beagle dogs. *Fundamental and Applied Toxicol.*, **16**: 667-686.

McManus, J.P. (1991) Metabolism of [<sup>14</sup>C] thiram in the rat: Urinary metabolite identification. Unpublished report from Uniroyal Chemical Company, Inc., Middlebury, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Matthiasck, L. (1973) Uber den Einfluss von L-cystein auf die Teratogenese durch Thiram (TMTD) bei NMRI-Mausen. *Arch. Toxikol.* **30**, 251-262. Submitted to WHO by UCB.

Musacchio, J.M., Goldstein, M., Anagnoste, B. Poch, G., & Kopin L.J (1966) Inhibition of dopamine beta-hydroxylase by disulfiram *in vivo*. *J. Pharmacol. Exp. Ther.*, **152**: 56-61.

Nomeir, A.A. & Markham, P. (1990) Disposition and metabolism of thiram in rats after pretreatment with thiram for 14 days. Unpublished report No. ADL 65492 from Arthur D. Little, Inc. Massachusetts, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Norris, K.J. (1989) Determination of volatile <sup>14</sup>C-residues from rats orally administered <sup>14</sup>C-thiram. Unpublished report No. 1113A from Analytical Development Corp., Colorado Springs, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Perocco, P., Santucci, M.A., Campani, A.G. & Forti, G.C. (1989) Toxic and DNA-damaging activities of the fungicides mancozeb and thiram (TMTD) on human lymphocytes *in vitro*. *Teratogenesis, carcinogenesis, and mutagenesis.*, **9**: 75-81.

Poth, A. (1990) *Salmonella typhimurium* reverse mutation assay with thiram. Unpublished report No. CCR 175116 from CCR Cytotest Cell Research GmbH & Co. KG, Rossdorf, Germany. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Prasad, H., Pushpavathi, K., Rita, P. & Reddy, P.P. (1987) The effect of thiram on the germ cells of male mice. *Fd. Chem. Toxic.* 25: 709-711.

Putman, D.L. (1987a) Chromosome aberrations in Chinese hamster ovary (CHO) cells. Unpublished report No. T5558.337 from Microbiological associates, Inc., Maryland, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Putman, D.L. (1987b): Micronucleus cytogenetic assay in mice. Unpublished report No. T5558.122 from Microbiological associates, Inc., Maryland, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Tesh, J.M., McAnulty, P.A., Willoughby, C.R. & Higgins, C. (1986) Thiram: Effects of oral administration upon pregnancy in the rat, preliminary teratology study. Unpublished report No. 86/TRK001/704 from Life Science Research, Suffolk, England. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Tesh, J.M., Ross, F.W. & Crisp, V.C. (1987) Thiram: Preliminary teratology study in the rabbit. Unpublished report No. 87/TRK003/122 from Life Science Research, Suffolk, England. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Tesh, J.M., McAnulty, P.A., Willoughby, C.R., Higgins, C., Wilby, O.K. & Tesh, S.A. (1988a) Teratology study in the rat. Unpublished report No. 87/TRK002/179 from Life Science Research, Suffolk, England. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Tesh, J.M., Ross, F.W., Crisp, V.C., Wilby, O.K. & Tesh, S.A. (1988b) Thiram: Teratology study in the rabbit. Unpublished report No. 87/TRK004/541 from Life Science Research, Suffolk, England. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Thouin, M.H. (1985a) Evaluation of the acute oral toxicity of TMTD technical in the rat. Unpublished report no.: 0174/238 from NOTOX v.o.f., s-Hertogenbosch, the Netherlands. Submitted to WHO by UCB.

Thouin, M.H. (1985b) Evaluation of the acute oral toxicity of TMTD technical in the rabbit. Unpublished report no.: 0113/211 from NOTOX v.o.f., s-Hertogenbosch, the Netherlands. Submitted to WHO by UCB.

Trutter, J.A. (1992) Oncogenicity study in mice with thiram. Unpublished report No. 798-223 from Hazleton Laboratories, Inc., 9200 Leesburg Turnpike, Vienna, Virginia 22182, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Weterings, P.J.J.M. (1985) Evaluation of the DNA repair inducing ability of TMTD technical in a primary culture of rat hepatocytes. Unpublished report No. NOTOX 0174/ER156 from NOTOX C.V. DD 's-Hertogenbosch, Netherlands. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Völkner, W. (1990) Mouse germ-cell cytogenetic assay with thiram. Unpublished report No. CCR 175127 from CCR Cytotest Cell Research GmbH & Co. KG, Rossdorf, Germany. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

Völkner, W. (1991) Mutation assay in somatic cells of the mouse (mouse spot test) with thiram. Unpublished report No. CCR 200902 from CCR Cytotest Cell Research GmbH & Co. KG, Rossdorf, Germany. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

WHO (1992) The WHO recommended classification of pesticides by hazard and guidelines to classification 1992-1993 (WHO/PCS/92.14). Available from the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland.

York, R.G. (1991) Two-generation reproduction study in rats. Unpublished report No. 399-104 from International Research and Development Corporation, Michigan, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.

York, R.G. (1992) Development toxicity study in New Zealand white rabbits. Unpublished report No. 399-121 from International Research and Development Corporation, Michigan, USA. Submitted to WHO by UCB Chemicals, Brussels, Belgium.