Thiram

(CAS No: 137-26-8)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

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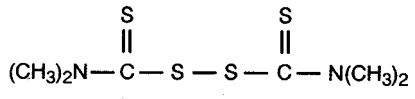
1 Introduction

The present document contains the assessment of the health hazard of thiram by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by I Gubbels-van Hal, M.Sc. (NOTOX BV, 's-Hertogenbosch, the Netherlands).

The evaluation of the toxicology of thiram has been based on the reviews by the American Conference of Industrial Hygienists (ACG99) and Edwards et al. in the 'Handbook of Pesticide Toxicology' (Edw91). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in September 1999, literature was searched in the on-line databases Medline, Toxline, and Chemical Abstracts, starting from 1965/66, and using the following key word: 137-26-8. Data of unpublished studies were generally not taken into account. Exceptions were made for studies that were summarised and evaluated by the FAO/WHO Joint Meeting of Pesticide Residues (FAO93). The final search was carried out in Toxline and Medline in December 2002.

In April 2003, the President of the Health Council released a draft of the document for public review. Comments were received by the following individuals and organisations: A Aalto (Ministry of Social Affairs and Health, Tampere, Finland) and M Laget (Taminco, Ghent, Belgium). These comments were taken into account in deciding on the final version of the document.

2 Identity

name	:	thiram
synonyms	:	tetramethylthiuram disulphide; tetramethylthioperoxydicarbonic diamide; bis(dimethylthiocarbamoyl) disulfide; TMT, TMTD, TMTDS.
molecular formula	:	$C_6H_{12}N_2S_4$
structural formula	:	
CAS number	:	137-26-8

3 Physical and chemical properties

molecular weight	:	240.4
melting point	:	155-156°C
boiling point	:	decomposes
flash point	:	89°C (closed cup)
vapour pressure	:	at 25°C: 2 x 10^{-3} Pa
solubility in water	:	at 25°C: 3 mg/100 mL
log $P_{octanol/water}$	:	1.73
conversion factors	:	not applicable

Data from ACG99, NLM02.

Thiram is a white to light yellow crystalline powder. It is reported to be stable under normal storage conditions, but may loose activity after prolonged exposure to air, heat, and moisture (ACG99, Dal88, NLM02).

4 Uses

Thiram is a protective fungicide applied in agricultural to foliage to control *Botrytis* species on grapes, lettuces, ornamentals, soft fruit, and vegetables. It also controls rust on ornamentals, scab, and storage diseases on apple and pear. In addition, it is used in seed treatments and as a repellent for rodents and certain large animals that cause damage to field crops. In agriculture, thiram is used as a wettable powder with up to 800 gram of active ingredient per kg (ACG99, Edw91, Lie94, NLM02). Thiram is also an ingredient of medicated soaps, suntan, and antiseptic sprays (Dal88). A major use of thiram is as a vulcanising accelerator in rubber processing (IARC91, NLM02). It is also used as a lubricating oil additive (ACG99).

According to the database of the Dutch Pesticide Authorisation Board (CTB)*, thiram is at present registered in the Netherlands for use as an active ingredient in fungicides and paints for specified applications.

* at: http://www.ctb-wageningen.nl/geel.html.

5 Biotransformation and kinetics

Human data

Following oral administration of thiram to human volunteers, CS_2 was detected in expired air (Lie94).

Animal data

Charles River rats (n=5/sex/dose level) were given ^{14}C-thiram (radiochemical purity: 100%) as single oral (gavage) doses of 1.9 or 125 mg/kg bw. At both doses, 25 and 3% of the administered radioactivity were excreted in the urine and faeces, respectively, within 7 days after dosing. At day 7, residues in tissues amounted to 3% of the administered radioactivity, most of it in blood, bone, and liver. Although excretion of radioactivity in expired breath was not determined, the author assumed that 70% of the administered thiram was expired as CO_2 or other volatile metabolites (Gay87). In another study, male Charles River rats (n=5) were given an oral (feed) dose of ^{14}C-thiram (purity: 92.4%) equivalent to 1.5 mg/kg bw. Within 72 hours after administration, 41% of the administered radioactivity was eliminated in expired air (CS_2 and CO_2), 38% in the urine, and 20% in the faeces and the gastrointestinal tract. At 72 hours after dosing, 6% of the administered radioactivity was retained in the carcass. The total recovery of radioactivity averaged 105%. From these data, it was concluded that 85% of the dose was absorbed into the systemic circulation of the rat, assuming that no absorbed radioactivity was eliminated via the bile into the faeces (Hil89).

Sprague-Dawley rats (n=5/sex) received a single oral dose of ^{14}C-thiram (radiochemical purity: >98%), following 14-day pre-treatment with non-radiolabelled thiram at a level of 2 mg/kg bw. Within 96 hours, 41-48, 35-40, and 2-5% of the dose were excreted in expired air, urine, and faeces, respectively. Most of the radioactivity in urine and expired air (amounts not specified) was excreted within the first 12 hours after dosing. In males and females, 83.7% and 89.6% of the administered dose was eliminated from the body, respectively, within 4 days. At 96 hours after dosing, traces of radioactivity were found in tissues, the highest levels in liver, kidneys, and blood cells, and the lowest in brain, plasma, and skeletal muscle. Total recovery was 85 and 93% for males and females, respectively (Nom90). The metabolism of thiram was studied in Charles River rats (n=3), following a single oral dose of 2.1 to 2.5 mg ^{14}C-thiram/kg bw. Within 3 days after administration, 25-43% of radioactivity was excreted in the

urine and an average of 61% in exhaled breath. The total radioactivity in urine and expired breath averaged 94% of the dose. Results indicated that metabolites in exhaled breath were mainly CO_2, CS_2, and COS. However, no characterisation or quantification of these metabolites could be made. Identification of metabolites in urine was not reported (Nor89). In another metabolism study, Charles River rats (n=2/sex) were given thiram via the diet at a dose equivalent to approximately 2.5 mg/kg bw/day for 9 weeks, followed by a single oral dose of ^{14}C-thiram. Within 24 hours after administration, approximately 60% of the radioactivity was eliminated in exhaled breath as CS_2 and 30% in urine. In the urine, 5 polar metabolites were identified: thiosulphenic acid (10% of the dose), an alanine conjugate of dimethyldithiocarbamate (9% of the dose), a glucuronide conjugate of dimethyldithiocarbamate (7% of the dose), an alanine derivate of CS_2 (3% of the dose), and the methyl ester of dimethyldithiocarbamate (2% of the dose). No unchanged thiram was detected in the urine. Identification of these metabolites demonstrated that thiram is biotransformed through reduction of the disulphide bond, leading to the formation of dimethyldithiocarbamate, and subsequent reactions of the thiol moiety to form oxidative and conjugative polar products (McM91). The metabolic pathway of thiram is shown in Figure 1 (see Annex I).

When male Sprague-Dawley rats were given intraperitoneal injections of 15, 30, or 60 mg thiram/kg bw, CS_2 was excreted in exhaled breath in amounts of 2.6, 26, or 120 nmol within 5 hours after administration (Dal86). In an older study, guinea pigs treated with single intraperitoneal doses of 10, 25, or 80 mg/kg bw excreted CS_2 in exhaled breath in amounts of 0.6 to 5.2% of the dose within 3 hours after treatment (Mer65).

6 Effects and mechanism of action

Human data

Excessive exposure to thiram may produce skin irritation with erythema and urticaria, conjunctivitis, mucous membrane irritation, and upper respiratory tract irritation (Dal88, Due87, NLM02).

Reports on allergic contact dermatitis have been published in workers in the rubber industry using thiram as a catalytic accelerator for the vulcanisation process of rubber (Gau57, Sch33), in subjects using medicated soap containing thiram (Bae54, Bla56), in subjects following contact with plants (Sau01) or a golf green (She64) sprayed with thiram-containing fungicides, and in workers

who had worn rubber gloves containing thiram (Ket84, Lis87). Contact dermatitis has also been reported in haemodialysed patients, probably by contact with rubber in haemodialysis equipment (Kru87, Pen76). More recently, a 41-year old woman developed acute exudative dermatitis after application of eardrops containing thiram to her dogs (Dwy97). In a retrospective study, the relative frequency of sensitisation to thiram was investigated in 2933 patients. Subjects were patch tested with thiram mix, comprising 0.25% tetraethylthiuram disulphide, 0.25% dipentamethylene disulphide, 0.25% tetramethylthiuram monosulphide, and 0.25% tetramethylthiuram disulphide, each in petrolatum. The observed frequency of thiram sensitisation was 2.8% (Knu96).

A case report of Henoch-Schönlein purpura was presented of a 23-year-old Mexican tree planter, handling seedlings treated with a solution of 42% thiram. Initial symptoms included abdominal pain, generalised arthralgias, and progressive purpuric rash. Later, he developed fever, malaise, severe nausea and vomiting, haematemesis, and melena. After hospitalisation, symptoms consisting of hypertension, palpable purpura, tenderness of the joints (thickened), abdominal pain, and black stool were noted. Haematological investigations showed an increased haemoglobin level and an increased erythrocyte sedimentation rate. A high serum IgA level, but a low serum albumin level was found. Leukocytoclastic vasculitis and granular deposits outlining or within the blood vessel walls were found at skin biopsy. Gastroduodenoscopy revealed a small Mallory Weiss tear and diffuse vasculitic erosions in the duodenum. The man recovered on corticosteroid therapy, but remained to have asymptomatic orthostatic hypotension (Due87).

In a group of 223 workers (42 men and 181 women), mostly aged between 20 and 50 years, engaged in the manufacture of thiram for more than 3 years, an excess of ocular irritation, coughing, thoracic pain, tachycardia, epistaxis, dermal lesions, myocardiodystrophia, liver dysfunction, and asthenia were reported compared to a unexposed control group of 193 persons. Thyroid gland disorders appeared 7-fold more frequently than in the control group. One case of a malignant lesion of the thyroid was reported in a subgroup of 105 thiram workers (IARC91).

In pregnant female operators who had been allegedly exposed to thiram during 30-90% of their working time, an increased incidence of late gestosis, a decreased urinary oestriol level (week 33-37), and an increased number of abortions and premature births were reported. Incidence and severity of these effects were correlated with duration of employment. Thiram exposure levels were not reported (And93).

Intolerance to alcohol has been observed in workers exposed to thiram, manifested by flushing of face, palpitation, rapid pulse, dizziness, and hypotension. These effects are thought to be due to increased blood acetaldehyde levels after alcohol consumption, caused by thiram-induced blocking of the metabolism of acetaldehyde (Edw91, Rei66).

Animal data

Irritation and sensitisation

Thiram is irritating to the skin and slightly irritating to the eyes of rabbits. Skin sensitisation in rabbits, and sensitisation by the subcutaneous and tracheal routes have been reported (ACG99, Edw91). No further details were given.

Acute toxicity

Results of acute lethal toxicity tests with thiram are summarised in Table 1.

Table 1 Summary of acute toxicity studies with thiram in experimental animals.

exposure route	species (strain; sex)	LC_{50}/LD_{50} (duration)	reference
inhalation	rat	300-1000 mg/m^3 (4 hours)	NLM02
	rat	>100 mg/m^3 [a]	Deb85
dermal	rat (Sherman; male, female)	>2000 mg/kg bw	Gai69
oral	rat	865 mg/kg bw	Leh51
	rat (Sherman; male)	640 mg/kg bw	Gai69
	rat (Sherman; female)	620 mg/kg bw	Gai69
	rat (Charles River ; male)	4000 mg/kg bw	Lee78
	rat (Charles River ; female)	1900 mg/kg bw	Lee78
	mouse (Charles River; male)	4000 mg/kg bw	Lee78
	mouse (Charles River; female)	3800 mg/kg bw	Lee78
	mouse (NMRI; female)	2300 mg/kg bw	Mat73
	rabbit	210 mg/kg bw	Wor87

[a] Exposure duration not given.

The oral LD_{50} values found for thiram show a broad range of values between and within species. A reason for these variations might be the use of different

formulations in the different experiments; however, no details on the composition of tested materials were provided.

Clinical signs of intoxication develop slowly and are characterised by rumpled fur and rapid breathing, and, then, ataxia, tremor, and dyspnoea. Death is preceded by convulsions (ACG99). Pathological examination after oral application revealed hyperaemia, focal ulceration of the gastrointestinal tract, focal necrosis of the liver and renal tubules, and patchy demyelination in the cerebellum and medulla (Dal88).

Short-term toxicity

Groups of 20 male CD rats received thiram via the diet at doses equivalent to 0, 30, 58, or 132 mg/kg bw for 13 weeks. Mortality occurred in 6/20 and 1/20 animals of the high- and mid-dose groups, respectively. Body weight gain and food intake were reduced dose dependently. No haematological abnormalities were reported, but clinical chemistry showed slight elevations in liver function tests (alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT)) at the high dose. In addition, microscopic examination also revealed moderate tubular degeneration of the testes with atypical spermatids in the epididymis in animals of the high-dose group. No statistical evaluation of the data was performed. The LOAEL was 30 mg/kg bw/day, based on reduced food intake and body weight gain (Lee78).

In another study, groups of 6 male Wistar rats were given thiram via the diet at doses equivalent to approximately 0, 11, 15, 22.5, 30, 45, or 60 mg/kg bw/day for 29 days. Pair-fed control groups (6 rats/group) were assigned to each of the 6 groups receiving thiram. For each parameter studied and each thiram dose, values for treated rats were compared to those for controls. Mean food intake and body weight were significantly reduced in a dose-dependent manner at 15 mg/kg bw/day and above. Absolute renal and testicular weights were significantly decreased at 45 mg/kg bw/day and above, and absolute seminal vesicle weights at 22.5 mg/kg bw/day and above. No haematological, clinical chemical, or histological data were shown. The NOAEL was 11 mg/kg bw/day, based on reduced food intake and body weight (Low80).

In an unpublished 13-week study, CD rats (n=10/sex/group) received thiram via the diet at doses equivalent to 0, 2.5, 25, or 50 mg/kg bw/day. At 50 and 25 mg/kg bw/day, body weights, body weight gains, and food consumption were significantly reduced in both sexes. In these dose groups, female animals had reduced haemoglobin, haematocrit, and red blood cell counts. White blood cell count was increased in both sexes, and clinical chemical tests showed decreased

total protein and albumin plasma levels and increased urea levels. A reduction in absolute liver weight and an increase in relative liver weight were also noted in these dose groups, compared to the control group. Macroscopic examination showed areas of erosion of the non-glandular stomach, and the mesenteric lymph nodes were diffusely red. Microscopically, the mucosa of the stomach had focal areas of ulceration and/or mucosal hyperplasia, accompanied by some submucosal inflammation and oedema. The NOAEL was 2.5 mg/kg bw/day (Keh88).

In an unpublished 28-day study, Crl:CD mice (n=10/sex/group) were given thiram via the diet at doses equivalent to 0, 54, 108, or 201 mg/kg bw/day for males and 0, 62, 118, or 241 mg/kg bw/day for females. Food consumption and body weights were reduced at all dose levels in a dose-related fashion. Males showed a dose-related decrease in haemoglobin, haematocrit, or red blood cell count at all dose levels. Females had increased platelet counts at the high- and mid-dose level. Relative brain, kidney, and liver weights were increased in a dose-related manner at all dose levels. The LOAEL was 54 mg/kg bw/day, the lowest dose tested (Keh89a).

In an unpublished 13-week study, beagle dogs (n=4/sex/group) were fed thiram at doses equivalent to 0, 2.2, 6.9, or 12 mg/kg bw/day for males and 0, 2.3, 7.3, or 13 mg/kg bw/day for females. At the high dose, food consumption and body weights were significantly reduced in both sexes. At all dose levels, red blood cell count and plasma total protein and albumin levels were reduced in both males and females. Haemoglobin and haematocrit levels were decreased in females at the high dose only. There were no treatment-related macroscopic or microscopic abnormalities. In this study, 2.2 mg/kg bw/day, the lowest dose tested , was a LOAEL (Keh89b).

In another unpublished study, beagle dogs (n=6/sex/group) were given thiram via the diet at dose levels equivalent to 0, 0.84, 2.6, or 7.4 mg/kg bw/day for males and 0, 0.9, 2.5, or 7.2 mg/kg bw/day for females, for 52 weeks. No signs of intoxication were noted. Ophthalmic examination at the end of treatment did not show abnormalities. At the high dose, red blood cell count was reduced in males and plasma total protein and albumin in both sexes. At 2.6 mg/kg bw/day, plasma total protein was lower in males only. Absolute and relative liver weights in males were significantly increased at 2.6 mg/kg bw/day and above. In females, relative liver weights were increased at the high dose only. No treatment-related macroscopic or microscopic changes were found. The NOAELs were 0.84 and 2.5 mg/kg bw/day for male and female dogs, respectively (Keh91a).

Beagle dogs (n=4/sex/group) received daily oral (capsule) doses of thiram of 0, 0.4, 4, or 40 mg/kg bw for 104 weeks. The dogs in the high-dose group had

severe signs of intoxication, including nausea, vomiting, excessive salivation, anorexia, occasional clonic convulsions, and decreased body weights, and all were subjected to unscheduled necropsy between weeks 21 and 29. The dogs had also ophthalmological changes such as fundal haemorrhage, miosis, and desquamation of the retina. Haematological and clinical chemical tests at week 13 of treatment showed a reduced haemoglobin, haematocrit, and red blood cell counts and increased in plasma alkaline phophatase, ALAT, and ASAT levels. At necropsy, 2 males and 3 females showed fatty degeneration, atrophy, and focal necrosis of hepatocytes at centrilobular and midlobular regions of the liver. Kupfer cell pigmentation (haemosiderin) was observed in 1 male and 2 females. Other effects observed included swelling and vacuolisation of the proximal tubuli of the kidney (2 females), follicular cell and C-cell hyperplasia of the thyroid (1 male and 1 female), and retina lesions (2 males and 3 females). At 4 mg/kg bw, no mortality occurred, but nausea, vomiting, and salivation were noted in both sexes, and 1 female showed convulsions from week 37 onwards. At the end of treatment, females had reduced haemoglobin, haematocrit, and red blood cell values compared to control animals. Plasma alkaline phosphatase and cholesterol were increased in both sexes and ALAT in females only. No changes in ASAT levels were noted. Absolute and relative liver weights were increased in males, but the weights of other organs in the treated groups of both sexes were comparable to those in the controls. Microscopic examination revealed hepatocellular degeneration in 2 males and 1 female. Swelling and vacuolisation of the proximal tubules of the kidney was seen in 2 females. Histological lesions in the central or peripheral nervous system were not found. No neoplastic lesions were observed in any of the animals at any dose level. The NOAEL was 0.4 mg/kg bw (Mai91).

The short-term toxicity studies are summarised in Table 2.

Long-term toxicity and carcinogenicity

Groups of rats (n=12/sex/group) were given dietary doses of thiram equivalent to 4.9, 15, 49, or 125 mg/kg bw/day for 65 weeks. The high dose was fatal to all rats within 17 weeks. No treatment-related mortality was observed in any of the other groups, but at 49 and 15 mg/kg bw, weakness, ataxia, and various degrees of paralysis of the hind legs were observed. Microscopic examination revealed calcification of the brain stem and cerebellum and dystrophic changes in the leg muscles. The NOAEL was 4.9 mg/kg bw/day (Leh52).

Table 2 Summary of short-term oral toxicity studies with thiram in experimental animals.

species (strain; sex)	dose (mg/kg bw/day)	exposure duration	critical effect	NOAEL (mg/kg bw/day)	reference
rat (Wistar; male)	0, 11, 15, 22.5, 30, 45, 60	29 d	reduced body weight	11	Low80
rat (Charles River; male)	0, 30, 58, 132	13 wks	reduced body weight gain	LOAEL: 30	Lee78
rat (Charles River; male, female)	0, 2.5, 25, 50	90 d	reduced body weight; anaemia (female); increased WBC; decreased plasma albumin and total protein; gastric mucosal irritation	2.5	Keh88
mouse (Charles River; male, female)	males: 0, 54, 108, 201 females: 0, 62, 118, 241	28 d	reduced body weight; anaemia (male); increased relative liver, kidney, brain weights	LOAEL: 54	Keh89a
dog (beagle; male, female)	males: 0, 2.2, 6.9, 12 females: 0, 2.3, 7.3, 13	90 d	reduced body weight; reduced RBC count; decreased plasma albumin and total protein	LOAEL: 2.2	Keh89b
dog (beagle; male, female)	males: 0.84, 2.6, 7.4; females: 0.90, 2.5, 7.2	52 wks	decreased plasma albumin and total protein; increased absolute, relative liver weights	males: 0.84 females: 2.5	Keh91a
dog (beagle; male, female)	0, 0.4, 4, 40	104 wks	anaemia (females); renal injury (females); liver injury	0.4	Mai91

Groups of CD rats (n=24/sex/group; controls: n=48/sex) were given dietary doses of technical thiram (purity: not given) equivalent to 5, 20, or 52 mg/kg bw/day for males and 0, 6, 26, or 67 mg/kg bw for females for 80 weeks. No significant differences in mortality were observed among treated and control groups. Body weights were significantly reduced in all male and female dose groups. Clinical signs of intoxication were lower body ataxia in 1 high-dose female and atrophy of both hind legs in another high-dose female between weeks 20 and 69. Six more females developed a similar ataxic syndrome, i.e., unusual gait with dragging of the hind feet and tail, between weeks 39 and 80. Eventually, paralysis posterior to the lumbar region and atrophy of the hind legs developed. In the high-dose and some mid-dose rats, patches or wide areas of alopecia occurred. No changes were observed in haematological or clinical chemical

parameters. At the end of treatment, relative thyroid and testes weights were increased in high-dose males and relative liver, spleen, kidney, thyroid, ovary, and brain weights in high-dose females. Microscopic examination revealed an increased incidence of squamous metaplasia of the thyroid in both sexes at the high dose and a dose-related increased incidence of fatty infiltration of the pancreas in females in all treated groups. Examination of the central and peripheral nervous systems did not reveal any specific lesions. The incidence and severity of nephritis were decreased in treated male or female rats when compared to controls. The incidences of tumours were not statistically significantly different between the treated and control groups. The LOAEL was 5 mg/kg bw/day, based on reduced body weight and mild fatty infiltration of the pancreas in females (Lee78).

Male and female Wistar rats (n=32/sex/group) received dietary doses of thiram (purity: 99.4%) equivalent to 0, 0.05, 0.5, 5, or 50 mg/kg bw/day for 2 years. No significant differences were noted in mortality between treated and control animals. In the male and female animals of the high-dose group, body weights were significantly decreased. Females showed a dose-related decrease in haemoglobin level and red blood cell count that was only statistically significant for haemoglobin at the high dose. A not statistically significant, dose-related decrease in haemoglobin levels was seen in males. No changes were found in white blood cell count of animals in treated groups when compared to the control group. Clinical chemistry tests showed a significant increase in plasma urea levels at the high dose in both sexes, while cholesterol was significantly reduced in males only. No changes were noted in plasma total protein, albumin, alkaline phosphatase, or ASAT levels. The activity of the enzyme carboxylesterase in plasma was significantly increased in females treated at 5 or 50 mg/kg bw/day. Although Knapek et al. did not further specify this effect, the committee considers it to be adverse. In male and female high-dose animals, relative heart, liver, and spleen weights were increased (significantly, except for male liver weight). Absolute kidney weight was significantly decreased in high-dose males and absolute brain weight in high-dose females, while absolute liver weights were increased in high-dose males and females (not statistically significant) and absolute heart and spleen weights in females (statistically significant). The incidence of macroscopic lesions was not statistically significantly different among the treated and control groups. Microscopic examination showed treatment-related lesions of the stomach mucosa in all groups. No relationship between thiram treatment and incidence of any tumour was observed. The NOAEL was set at 0.5 mg/kg bw, based on changes in carboxylesterase activity at higher doses (Kna89). According to the committee, the final evaluations were

done with only 8 animals/sex, which was considered to be too low to reach statistical power. It is not clear from the report whether the effects reported were found in animals surviving until study termination or in interim killed animals. According to the committee this report was insufficiently documented.

In another 2-year rat study, Wistar rats (n=64/sex/group) received dietary doses of thiram (purity: 98.7%) equivalent to 0, 0.1, 1.2, or 11.6 mg/kg bw for males and 0, 0.1, 1.4, or 13.8 mg/kg bw for females. The mortality of females was dose-dependently increased in the 13.8- and 1.4-mg/kg bw groups during the last 8 weeks of the treatment period. Body weight gain and food consumption were decreased in both males and females of the high-dose group. During the last part of the test period, food consumption was also decreased in males receiving 1.2 mg thiram/kg bw. In females of the high-dose group, haemoglobin and haematocrit levels and red blood cell count were significantly decreased at week 26, but no differences were found at termination (week 104). At week 104, slightly but significantly increased plasma ALAT levels were found in all treated groups (both sexes) and a slightly, significantly increased plasma ASAT level in high-dose males and in mid-dose females. No changes were observed in plasma alkaline phoshatase and urea levels. The committee considered the liver enzyme changes to be incidental to treatment. At termination, absolute kidney weight, and absolute and relative calf muscle (*M. triceps surae*) weights were decreased in high-dose males. Females showed significantly increased absolute thyroid weights in all treated groups, but significantly decreased absolute liver, kidney, and calf muscle weights at the high dose only. Microscopic examination revealed a significant increase in atrophy and degeneration of this muscle in females of the high-dose group. This effect was considered to be secondary to the atrophy and degeneration of the sciatic nerve that was also observed in high-dosed females (n=15). Pituitary adenomas were observed in 4/5 mid-dose and 7/8 high-dose females, which were found dead during the last 8 weeks of the treatment. Since the incidence of these tumours in these 2 female groups at terminal kill was less than in the low-dose or control groups, overall incidences of the tumour were comparable in all groups. In addition, this type of tumour is often seen in ageing rats, and, therefore, the committee did not consider these tumours to be related to thiram treatment. The incidence of fibroadenomas of the mammary gland in females was significantly decreased in a dose-related fashion in the high-dose group. The incidences of other tumours were comparable to those of controls in both sexes. No evidence for carcinogenic effects of thiram was found. The NOAEL for long-term toxicity was 1.2 or 1.4 mg/kg bw/day for males or females, respectively (Mai91).

In an unpublished study, groups of rats (SD; n=60/sex/group) were given dietary doses of thiram (purity: 97.5%) equivalent to 0, 1.5, 7.3, and 15 mg/kg bw for males and 0, 1.8, 8.9 and 19 mg/kg bw for females for 104 weeks. Compound-related clinical signs included swollen nose, soft faeces, and opaque eyes in the high- and mid-dose animals. At these doses, body weight and body weight gain were significantly decreased in both sexes. Food consumption was significantly lower in all treated groups compared to controls. At the high and mid dose, red blood cell count and haemoglobin and haematocrit levels were decreased in female rats, compared to controls. In high- and mid-dose male rats, extramedullary haematopoiesis of the liver and steatosis and multifocal acinar atrophy of the pancreas were observed. In high-dose females, extramedullary haematopoiesis and bile duct hyperplasia were reported. Steatosis of the pancreas was noted in females receiving 19.5 and 8.9 mg/kg bw/day. Microscopic examination did not reveal treatment-related neurological lesions. There was no statistically significant increase in the incidence of any tumour in any of the treated groups, compared to the control group. Kehoe concluded that thiram was not carcinogenic up to doses of 15 and 19 mg/kg bw in males and females, respectively. The NOAEL was 1.5 or 1.8 mg/kg bw for males or females, respectively (Keh91b).

In a carcinogenicity study, groups of F344 rats (n=50/sex/group) were fed thiram (purity: not given) at doses equivalent to 0,18, or 39 mg/kg bw for males and 0, 20, or 42 mg/kg bw for females for 104 weeks. All surviving rats were sacrificed at week 112. No differences in survival between the treated and control groups became apparent in males, but the high-dose females had shorter survival. Body weight gain and food intake were significantly decreased at the high dose, especially in females. Red blood cell count was significantly reduced in the high-dose males, and white blood cell count was decreased in females at both dose levels. Hasegawa et al. considered these changes not to be treatment related. Clinical chemistry liver function tests showed slight liver damage in males (not further specified). Macroscopic and microscopic examination did not show an increased incidence of neoplastic or non-neoplastic lesions in any tissue at any of the dose levels. The incidence of leukaemia in both sexes was statistically significantly reduced in a dose-related fashion in both treated groups, compared to the control group. The incidence of pituitary chromophobe adenomas in females was significantly lower in both treated groups and that of C-cell adenomas of the thyroid was significantly lower in high-dose females. 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. The NOAEL was 18 mg/kg bw/day based on reduced body weight gain (Has88).

In a carcinogenicity study, 2 hybrid strains of mice (the offspring of C57BL/6 females and C3H/Anf or AKR males) (n=18/strain/sex) were fed thiram (purity: not given), beginning at 7 days of age, for 18 months. Until weaning, the compound was given at doses of 10 mg/kg bw/day by stomach tube, thereafter via the food at doses equivalent to 4.2 mg/kg bw/day. No increased incidence of tumours was found in any sex-strain subgroup or in the combined sexes of either strain compared to untreated control mice (Inn69).

In an unpublished study, CD mice (n=50/sex/group were given dietary doses of thiram (purity, 97.5%) of 0, 3, 24, or 50 mg/kg bw/day for males and 0, 3, 57, or 112 mg/kg bw for females for 97 weeks. No treatment-related increase in mortality was observed. The only observed clinical signs of toxicity were sores and redness of the skin (mainly of the ears), consistent with bacterial dermatitis, in the high- and mid-dose male mice and in the high-dose female mice. A dose-related significant decrease in body weight, body weight gain, and food consumption was observed in the mid- and high-dose male and female mice. Significant decreases in red blood cell count, haemoglobin, and haematocrit values were found in the high-dose females. Macroscopic and microscopic examination did not show an increased incidence in neoplastic lesions in any of the treated groups compared with the control group. Non-neoplastic lesions in the mid- and high-dose male and female mice included retinal atrophy, intracytoplasmic protein-like droplets in the epithelium of the urinary bladder, and necrosis and inflammation of the skin. Increased pigment in the spleen and decreased pigment in the inner adrenal cortex was seen in mid- and high-dose female mice and hyperkeratosis of the non-glandular stomach in mid- and high-dose females and in high-dose males. It was concluded that thiram is not carcinogenic up to 50 mg/kg bw/day for male mice and up to 112 mg/kg bw/day for female mice. The NOAEL was 3 mg/kg bw (Tru92).

The tumour-initiating and tumour-promoting potency of thiram were tested in Swiss albino mice by the 2-stage skin carcinogenic model. Six groups of male mice (n=15/group) were topically treated in the following way. In group 1, animals received a single dose of 1 mg thiram in 0.2 mL DMSO. Two weeks thereafter, croton oil (a promotor) was applied for 6 weeks, twice weekly. In group 2, animals received a single dose of 10 mg thiram in 0.2 mL DMSO. Croton oil was applied as in group 1. In group 3, animals received a single dose of 7,12-Dimethylbenz[a]anthracene (DMBA), a known initiator of skin carcinogenesis, in 0.2 mL DMSO without further treatment. In group 4, animals were given a single dose of DMBA in 0.2 mL DMSO. Croton oil was applied as in groups 1 and 2. Group 5 received a single dose of DMBA in 0.2 mL DMSO.

2 weeks thereafter, thiram (1 mg/0.2 mL DMSO) was applied for 6 weeks, twice weekly. Group 6 received croton oil for 6 weeks, twice weekly. The positive control DMBA induced skin tumours in 13 of 15 mice, when DMBA application was followed by treatment with croton oil (group 4). No skin tumours developed when DMBA application was followed by treatment with thiram (group 5) or when DMBA was applied without further treatment (group 3). One mouse bearing a skin tumour was found after treatment with thiram at 10 mg, followed by croton oil treatment (group 2). No skin tumours were found in animals treated with thiram at 1 mg, followed by croton oil treatment (group1), or when croton oil was applied without further treatment (group 6). Thus, there was no significant increase in skin tumours in the thiram-treated groups, either as initiator or promotor (Geo95).

A summary of the long-term toxicity of thiram is shown in Table 3.

Table 3 Summary of long-term oral toxicity/carcinogenicity studies with thiram in experimental animals.

species (strain; sex))	dose (mg/kg bw/day)	exposure duration	critical effect	NOAEL (mg/kg bw/day)	reference
rat (male, female)	0, 4.9, 15, 49, 125	65 wks	ataxia; paralysis of legs	4.9	Leh52
rat (CD; male, female)	males: 0, 5, 20, 52 females: 0, 6, 26, 67	80 wks	reduced body weight; pancreas injury	LOAEL: 5	Lee78
rat (Wistar; male, female)	0, 0.05, 0.5, 5, 50	2 y	reduced haemoglobin (females); changes in absolute and relative organ weights carboxyesterase	5 0.5	Kna89
rat (Wistar; male, female)	males: 0, 0.1, 1.2, 11.6 females: 0, 0.1, 1.4, 13.8	2 y	reduced body weight; increased mortality; anaemia (females) nerve degeneration and muscle atrophy (females)	1.2	Mai91
rat (SD; male, female)	males: 0, 1.5, 7.3, 15 females: 0, 1.8, 8.9, 19	2 y	anaemia (females); reduced body weight; pancreas and liver injury	1.5	Keh91b
rat (F344; male, female)	males: 0, 18, 39 females: 0, 20, 42	2 y	reduced body weight	18	Has88
mouse (C57BL/6xC3H/Anf; C57BL/6xAKR; male, female)	0, 4.2	78 wks	-	4.2	Inn69
mouse (CD ; male, female)	males: 0, 3, 24, 50 females : 0, 3, 57, 112	97 wks	reduced body weight	3	Tru92

Based on the above data, the committee concludes that thiram unlikely has carcinogenic potential.

Mutagenicity and genotoxicity

Mutation assays comprised tests for the detection of gene mutations in bacteria, in mammalian cells, and in *Drosophila* (*in vitro*), clastogenic effects, e.g., sister chromatid exchanges (SCE), chromosomal aberrations, and micronuclei *in vitro*, and dominant lethal mutations and micronuclei *in vivo*, and other genotoxicity assays, e.g., tests for DNA damage and repair (*in vivo* and *in vitro*).

- *In vitro* tests:
  - Gene mutation assays. Tests for reverse mutations were positive in *S. typhimurium* strains TA100 and TA1535 at concentrations in the range of 10 to 120 µg/plate or 10 to 1000 µg/plate, without or with metabolic activation by a rat liver microsomal S9 fraction, respectively (And80, Cre92, Hed79, Mor83, Pot90, Zdz79). No increased mutation rates were found in strains TA98, TA102, TA1537, and TA1538 at concentrations up to 1000 µg/plate with or without metabolic activation (And80, Cre92, Mor83, Pot90). However, one author found positive results in strains TA98 and TA1538 at a concentration of 100 µg/plate in the presence of S9 (Zdz79). When tested in *E. coli* strain WP2 *uvrA*, thiram induced reverse mutations at concentrations in the range of 15 to 60 µg/plate in the absence or presence of S9. However, under these conditions, the test was negative in *E. coli* strain WP2 (Cre92).
    Thiram did not induce gene mutations in the *hprt* forward mutation assay in cultured Chinese hamster V79 lung cells, when tested at concentrations up to 1.6 µg/mL in the presence of S9 (Don83). In another study, negative results were obtained when tested at concentrations in the range of 1-10 µg/mL or 10-56 µg/mL without or with metabolic activation, respectively (Deb86). It was positive when tested at a cytotoxic concentration of 10 µg/mL without metabolic activation (Pas85).
    Thiram induced sex-linked recessive lethal mutations in *D. melanogaster* at concentrations in the range of 1-5 mg/mL. The mutagenicity was manifested in the spermatid/spermatocyte stage (3 to 6 days after treatment), and no increased mutation rate was found in the pre-meiotic stages (6 to 9 days after treatment) (Don83).
  - Cytogenicity assays. Thiram induced a dose-relatedly increased frequency of SCEs in cultured human lymphocytes, at concentrations in the range of

1.2 to 1200 μg/L in the absence or presence of S9. At the high dose, a 2-fold increase over the frequency of SCEs in controls was seen (Pie90). A significant dose-dependent increase in SCEs was also found when cultured human lymphocytes were treated with thiram concentrations ranging from 5 to 25 μg/mL with metabolic activation. However, negative results were found in the absence of S9, probably due to the high cytotoxicity at 15 μg/mL and higher (Per89). In another study, thiram did not induce an increased SCE frequency in cultured Chinese hamster ovary (CHO) cells at concentrations in the range of 24 to 240 μg/L without S9, or 24 to 2400 μg/L with metabolic activation (Don83).

The frequency of chromosome aberrations in cultured CHO cells remained unaffected following treatment with 3-23 μg/L in the absence of S9, or with 200-1500 μg/L in the presence of metabolic activation (Put87a). However, positive results were obtained in a chromosomal aberration assay (excluding gaps) in cultured CHO cells at concentrations ranging from 7.5 to 34.8 μg/L in the presence but not in the absence of S9. The increase was not dose dependent (Mos94). A dose related increased incidence of micronuclei in cultured human lymphocytes was observed in the dose range 0.5-24 μg/mL in the absence or presence of S9 (Vil98).

- Other assays. Thiram induced unscheduled DNA synthesis in cultured human lymphocytes when tested at concentrations of 5-50 μg/L and higher, in the presence but not in the absence of metabolic activation. The increase was not dose dependent, probably due to cytotoxicity at the higher dose levels (Per89).

  When primary rat hepatocytes were treated with thiram at concentrations ranging from 30 to 10000 μg/L, no effect on DNA repair was demonstrated (Wet85).

  There was no evidence of alkylation activity of thiram towards the nucleophiles 4-(*p*-nitrobenzyl)-pyridine or deoxyguanosine (Hem80).

  Thiram caused a statistically significant increase in the frequency of single strand DNA breaks and alkali labile sites in cultured human testicular cells at a concentration of 24 μg/mL without metabolic activation. In cultured rat testicular cells, a statistically significant increase of DNA damage was demonstrated at concentrations in the range of 7.2-72 μg/mL (Bjø96). DNA damage (single strand DNA breaks) was also demonstrated in cultured human lymphocytes at thiram concentrations in the range of 0.1-8 μg/mL in the presence or absence of S9 (‘Comet’ assay) (Vil98).

- *In vivo* tests:
  - cytogenicity assays. In a dominant lethal mutation assay, 20 male mice were fed thiram (purity: 75%) doses equivalent to 150 mg/kg bw/day for 8 weeks (the entire spermatogenesis cycle). Following mating, pregnant mice were sacrificed on day 14 of gestation. A significant increase in dead implants compared to controls was found, indicating a thiram-induced dominant lethal effect (Agr97).
    When thiram (purity: 99.7%) was orally (gavage) administered to male B6C3F1 mice (n=3/group) at 0, 100, 300, and 900 mg/kg bw for 4 consecutive days, or at 300 mg/kg bw for 8 and 12 days, no statistically significant increased incidences of micronuclei in isolated splenocytes were found at 24 hours after the last treatment (Vil98).
    No increased incidence was found in bone marrow cells of Chinese hamsters (n=1-2/sex/group), 30 hours after single intraperitoneal injections of 0, 100, 200, or 500 mg thiram/kg bw (Don83).
    When F1 hybrid mice (n=15/sex) were treated with a single intraperitoneal dose of thiram (purity: 80%) of 100 mg/kg bw, a significant increase in the incidence of micronuclei in bone marrow polychromatic erythrocytes was found at 24 hours after treatment (Pas85).
    In another study, B6C3F1 mice (n=5/sex/group) were given single intraperitoneal injections of thiram (purity: 99.7%) of 0, 12.5, 25, or 50 mg/kg bw. In a second experiment, a dose of 37.5 mg/kg bw was given. No increased incidence of micronuclei was found in bone marrow cells of either sex, collected at 24 hours after treatment. However, a statistically significant increase was found in bone marrow cells of male mice at 25 mg/kg bw and above, at 48 hours after treament (Cre92).
    When thiram (purity: 75%) was administered to male Swiss albino mice (n=6/group) as single intraperitoneal doses of 0, 25, 50, or 100 mg/kg bw, a statistically significantly increased incidence of micronuclei in polychromatic erythrocytes was found, either at 30 or 48 hours after treament (Agr97).
    In an unpublished study, the frequency of micronuclei was not significantly changed in CD-1 mice, treated with thiram (purity: 99.7%) at single intraperitoneal injections of 38 to 377 mg/kg bw (Put87b).
    Male Swiss albino mice (n=3-5/group) were intraperitoneally treated with thiram (purity: not given) at doses of 0, 100, 150, or 200 mg/kg bw. The treatment was repeated after 24 hours. No increased incidence of micronuclei in bone marrow cells were observed 24 hours after the last treatment (Geo95). The effect of thiram at the germ cell level was studied

in groups Swiss albino mice, given thiram at doses of 0, 80, 200, or 320 mg/kg bw for 3 days by gavage. A significant, dose-dependent increase in the frequency of chromosomal polyploidy in primary spermatocytes was found (Pra87).

- Other assays. In a 'Comet' assay, thiram induced DNA damage, i.e., single strand breaks or alkali labele sites were found in lymphocytes of rats 24 hours following oral treatment with 300 mg/kg bw/day for 8 and 12 days. Negative results were obtained in splenocytes (Vil98).

In summary, *in vitro*, thiram induced gene mutations in sensitive strains of *S. typhimurium* and *E. coli* and in mammalian cells at cytotoxic concentrations. DNA damage was demonstrated in mammalian cultures, but equivocal results were obtained for cytogenetic effects. *In vivo*, thiram induced dominant lethal mutations and germ cell abnormalities in mice, but no significant increase in the frequency of micronuclei in bone marrow cells was observed after repeated high oral doses. Data on the induction of micronuclei in bone marrow cells of mice treated by intraperitoneal injection at high doses were conflicting. DNA damage was demonstrated in lymphocytes of rats at high oral doses.

Based on these data, the committee concludes that thiram exhibits mutagenic and genotoxic potential *in vitro*. *In vivo*, clastogenic effects were only observed at levels close to the maximum tolerated dose.

Reproduction toxicity

Groups of weanling male CD rats (n=20/group) were given technical-grade thiram (purity: not given) via the diet at daily doses equivalent to 0, 30, 58, or 132 mg/kg bw for at least 13 weeks before mating with untreated females. In high-dose males, mortality occurred in 70% of the animals. Body weights were reduced in a dose-dependent fashion in all treated groups. At the high- and mid-dose levels, animals lost hair and had rough coats. At 132 mg/kg bw/day, thiram induced infertility as shown by the inability to fertilise females. Microscopic examination revealed testicular hypoplasia, tubular degeneration, and atypical spermatids in the epididymides. The NOAEL for effects on the male reproductive system was 58 mg/kg bw/day (Sho76). In several studies, thiram induced morphologically abnormal sperm in mice. Abnormal sperm heads were observed in CFWx C57BL mice, 5 weeks after single intraperitoneal injections of thiram at 50 or 100 mg/kg bw or after repeated injections at 30 mg/kg bw/day for 5 days. No abnormalities were found at 1 week after treatment (Zdz82). A dose-dependent increase in sperm head abnormalities was observed in Swiss

albino mice treated with thiram at doses of 80, 200, or 320 mg/kg bw for 3 days by gavage (Pra87). In a more recent study, sperm abnormalities were investigated in Swiss mice (n=6/group) after treatment with thiram (purity: not given) as single intraperitoneal doses of 500 or 1000 mg/kg bw or as daily intraperitoneal doses of 250 mg/kg bw for 5 days. One month after the end of treatment, a significant dose-related increased incidence of sperm abnormalities was observed, consisting of sperm with acrosomes bent upward, acrosomes bent downward, or without acrosomes. Head abnormalities, like double heads, banana heads, amorphous heads, microcephaly, and macrocephaly were found. Instances of double tails and coiled sperm were also recorded. The incidences of sperm abnormalities in mice receiving a single dose of 1000 mg/kg bw or 5 repeated doses of 250 mg/kg bw /day were comparable (Hem93). B6C3F1 mice were treated intraperitoneally with thiram (purity: 99%) at a single dose of 75 mg/kg bw (32 animals) or repeated doses of 25 mg/kg bw/day for 5 days (24 animals). Mortality was observed within 5 days in 29% and 15 % of the animals, respectively. Reproductive indicators were assessed at days 14, 28, 35, and 56 after the end of treatment. No effects were observed on body weight, testes weight, testicular sperm head numbers, or activities of specific testicular enzyme levels at any time after administration. The authors conclude that under the conditions of the study, thiram did not cause cytotoxicity on differentiating spermatogonia or on late spermatocyte stages of mice gonads (Tra94).

In 2 separate studies, male rats (n=25/group) received thiram (purity: not given) at oral (gavage) doses of 5, 10, and 25 mg/ kg bw in peanut oil for 90 days or 360 days, respectively. In both studies, a dose-related increased mortality was observed. Clinical signs observed in both studies consisted of diarrhoea, salivation, nasal bleedings, and ataxia. Body weight gain was reduced in high-dose males in the 90-day study and in a dose-dependent way in all treatment groups in the 360-day study. Relative testes weight was significantly increased in the 90-day study at 25 mg/kg bw and in the 360-day study at 25 and 10 mg/kg bw. In the latter study, the relative epididymis weight was significantly increased, and the relative weights of seminal vesicles and prostate were significantly decreased at 25 and 10 mg/kg bw, compared to controls. Microscopic examination revealed inactive sperm cells, necrosis of seminiferous tubules with enlarged interstitium, detachment of germinal cells, and accumulation of debris in the lumen of the tubules. The activities of testicular enzymes lactate dehydrogenase, alkaline phosphatase, and glucose-6-dehydrogenase were significantly increased and that of succinate dehydrogenase and acid phosphatase significantly decreased at 10 and 25 mg/kg bw in the 90-day and in all treatment groups in the 360-day study. Testicular ATPase activity was increased in all dose

groups in the 360-day study and testicular free sialic acid at all treatment groups in both studies. Serum cholesterol was increased and testicular protein was reduced in all treated groups in the 360-day study (not determined in the 90-day study). The NOAEL was 5 mg/kg bw/day for the
90-day study, based on changes in activity of testicular enzymes. For the 360-day study, a LOAEL of 5 mg/kg bw/day was established, based on decreased body weight and changes in activity of testicular enzymes (Mis94, Mis98).

The effect of thiram on female reproduction was examined in female CD rats (n=20/group), given daily dietary doses of technical-grade thiram (purity: not given) of 0, 30, or 96 mg/kg bw for periods of time ranging from 14 days to 4.5 weeks, prior to mating with non-treated males. After mating, all females were fed the control diet. At the end of the 2-week dosing period, mean body weight and food consumption were significantly decreased in the high-dose animals. The number of implants and pups per dam were reduced in low-dose dams. No further results on reproductive parameters were shown. At the high dose, the dioestrous phase of the oestrus cycle was delayed. This effect was reversible after 9 days without thiram. The LOAEL was 30 mg/kg bw/day, the lowest dose tested (Sho76).

Another study was conducted to investigate the mechanism of the acute effects of thiram on the hormonal control of ovulation in the rat. A key endocrine event in ovulation is the pre-ovulatory surge of luteinising hormone (LH), which occurs in the rat during a restricted sensitive period of time just prior to the appearance of the LH surge, typically between 4 pm and 7 pm. To establish the effect of thiram on the LH surge, ovariectomised oestrogen-primed Long-Evans rats were treated with thiram at intraperitoneal doses of 0, 6, 12, 25, 50, or 100 mg/kg bw. At 100 and 50 mg/kg bw, thiram completely blocked the LH surge in all rats tested, while doses of 25 and 12 mg/kg bw blocked the surge in 75% and 40% of the treated animals, respectively. The NOAEL was 6 mg/kg bw. In a second experiment, intact rats were intraperioneally injected with 0, 12, 25, and 50 mg thiram/kg bw at 1 pm on the day of vaginal pro-oestrus. Thiram at 50 mg/kg bw blocked the LH surge in all rats examined on the afternoon of vaginal pro-oestrus, while 25 mg/kg bw blocked the surge in 60% of the females tested. The NOAEL was 12 mg/kg bw. No influence of thiram on oestradiol levels was reported. Examination of the oviducts 48 hours after the pro-oestrous injection of 50 mg/kg bw at 1 pm revealed that all rats had ovulated, i.e., ovulation was delayed for 24 hours. The conclusion was that thiram is able to block the LH surge and inhibits subsequent ovulation if administered during a sensitive period prior to the initiation of the surge. According to Stoker et al., thiram most probably inhibits noradrenaline synthesis by suppressing the activity of

dopamine-β-hydroxylase. Because of a decreased noradrenaline level in the hypothalamus, the release of gonadotropin-releasing hormone, and indirectly the release of LH, are lowered (Sto93). In a subsequent study, the effect of a thiram-induced delay in ovulation on pregnancy outcome in the rat was investigated. Groups of female rats were treated with a single intraperitoneal injection of thiram (50 mg/kg bw) at 1 pm on the day of vaginal pro-oestrus and paired the following evening (approximately 30 hours later) with untreated males (thiram-delayed females). A thiram-treated, non-delayed and an untreated group of females were mated on the same day, 6 hours after treatment. A significant decrease in the proportion of pregnant females and the litter size on gestational day 20 and a significant increase in the number of resorptions were seen in the thiram-delayed group, but not in the non-delayed group. According to Stoker et al., this indicated that thiram-induced delay in ovulation and not the exposure to thiram per se was responsible for altered pregnancy outcome. On gestational days 7, 11, and 20, the number of implantations in the delayed females was not statistically significantly different from control and non-delayed females. However, the number of live embryos was reduced on gestational day 11 in the delayed females. The mean developmental score, head length, crown-rump length, and somite number in the delayed group was also reduced, indicating retarded development of live embryos. According to Stoker et al., the results demonstrate that thiram-induced delayed ovulation does not alter the number of oocytes released or the number of implants. Effects on the concepti from these females occur during mid-gestation (Sto96).

In an unpublished 2-generation reproduction toxicity study, 63-days-old Crl:CD rats (n=26/sex/group) were given thiram (purity: 97.6%) via the diet at levels equivalent to 0, 1.5, 2.9, or 8.9 mg/kg bw/day for males and 0, 2.3, 4.6, or 14 mg/kg bw/day for females for 81 days prior to mating. Three litters (F1a, F1b, and F1c) were produced, and F1c rats were fed diets containing thiram at levels equivalent to 0, 1.8, 3.8, or 11 mg/kg bw /day for males and 0, 2.4, 5.1, or 16 mg/kg bw/day for females for at least 105 days after weaning, before being bred to produce 2 litters (F2a and F2b). High-dose females showed decreased body weights during gestation and lactation of F1 and F2 offspring. Mean maternal body weight was also decreased at 4.6 mg/kg bw/day during the F1a-gestation period. Food consumption was reduced in high- and mid-dose F0 males and females. Mean offspring body weights were significantly reduced in all litters across both generations at the high dose. No treatment-related clinical signs of toxicity were observed, and no treatment-related abnormalities were seen in male and female fertility indices, gestation length, litter size, numbers of viable and

stillborn pups, and offspring survival and growth during lactation. The reproductive NOAEL was 4.6 and 2.9 mg/kg bw for males and females, respectively, based on reduced body weights in offspring. The parental NOAEL was 2.3 and 1.5 mg/kg bw/day, based on reduced food intake (Yor91).

Developmental toxicity studies were conducted in rats, mice, and rabbits. Pregnant CD rats (n=7-18/group; controls: n=28) received oral (gavage) doses of technical-grade thiram (purity: not given) of 40, 90, 136, or 164 mg/kg bw/day on gestational days 6 to15; a dose of 200 mg/kg bw was given on gestational days 7 to 12. Animals were sacrificed on gestation day 20. Mortality was 67% in the high-dose group. Food consumption and body weight gain during gestation were reduced in all treated groups compared to controls. Litter size was decreased at doses 136 mg/kg bw. The number of implants per dam was significantly decreased at 164 and 200 mg/kg/day. At 136 mg/kg bw and above, the number of live fetuses was significantly decreased, and a corresponding increase in resorptions was seen, amounting to 100% at 164 and 200 mg/kg bw. Fetal body weights were significantly reduced in all treated groups. At 136 mg/kg bw, anomalies including domed cranium, hydrocephalus, unossified sternebrae, and incomplete ossified supraoccipital were observed. The committee considered these changes the result of maternal toxicity. The LOAEL for maternal as well as for fetotoxicity was 40 mg/kg bw/day. For teratological effects, a NOAEL of 90 mg/kg bw was established (Sho76).

In an unpublished study, groups of 25 pregnant CD rats received oral (gavage) doses of thiram (purity: 99.8%) of 0, 7.5, 15, and 30 mg/kg bw/day on gestational days 6 to 15. Animals were sacrificed on day 20 of gestation. No deaths were observed and the only clinical sign observed was a dose-related increase of alopecia on various parts of the body. A transient, dose-related loss of maternal body weight was observed at 15 and 30 mg/kg bw/day. Body weight gain was significantly reduced during the treatment period in females of the low-dose group, but subsequent weight gain was similar to that of the controls. No effects on implantation or fetal survival were reported. Fetal weights were decreased at 15 and 30 mg/kg bw/day (reaching statistical significance at 30 mg/kg bw). Placental weights were significantly decreased at all dose levels. Fetal immaturity was observed at 30 mg/kg bw/day, and an increased incidence of thirteenth ribs of reduced size was observed at 15 and 30 mg/kg bw/day. These effects were considered a result of maternal toxicity. The NOAEL for developmental effects was 7.5 mg/kg bw. For maternal effects, a LOAEL of 7.5 mg/kg bw/day was established (Tes88a).

Pregnant NMRI mice (n=19-23/group) received oral (gavage) doses of thiram (purity: not given) of 0, 357, and 714 mg/kg bw/day on gestational days 6 to 17. Treatment-related maternal mortality or signs of toxicity were not reported. No differences in the number of implantations per female were observed between the treated and the control groups at any dose level. A dose-dependent decrease in the frequency of life fetuses at birth and a corresponding increase in the frequency of early and late resorptions and of fetal deaths were seen. These changes were statistically significant at the high dose only. Fetal body weight was significantly decreased in the high-dose group. An increased frequency of fetal malformations was seen at both doses compared to controls, especially at the high dose (55.2% vs. 0.9%). Dose-dependent fetal malformations were characterised by wavy ribs, cleft palate, distorted bones, and micrognatia. Retarded fetal development was also observed at the high dose. In a parallel experiment, pregnant NMRI mice were treated with thiram on specific days of gestation in order to find out the most susceptible phase of embryonic development. Pregnant SW mice were used for comparison. NMRI and SW mice were given oral doses of 357, 714, and 1071 mg/kg bw, and 250, 500, 1000, and 1500 mg/kg bw, respectively, on gestational days 10 and 11, or 12 and 13. The 12th and 13th day of embryonic development, i.e., the end of embryonal and the beginning of the fetal development phase, proved to be the most susceptible phase. Embryotoxic effects in NMRI and SW strain were comparable and increased in a dose-dependent fashion. At doses of 10 mg/kg bw/day and higher, NMRI mice were more susceptible to the induction of cleft palates and SW mice to the induction of wavy ribs. A NOAEL for developmental effects in SW mice was 250 mg/kg bw/day. For NMRI mice, a LOAEL was 357 mg/kg bw/day was established (Rol71).

In another study, pregnant Swiss-Webster mice (n=18-19/group) received oral (gavage) doses of technical-grade thiram (purity: not given) of 0, 100, or 300 mg/kg bw/day on gestational days 6 to 14. Animals were sacrificed on gestational day 18. Mortality was 22% in the high-dose group. The treatments did not significantly change body weight gain during gestation, litter size, number of implants per dam, number of live fetuses, or incidence of resorptions. No significant differences in body weights of fetuses from treated or control dams were found. At the high dose, anomalies including hydronephrosis, collapsed cranium, thick atrium wall, malaligned sternebrae, and the presence of fibrous material connecting lens and retina were observed. No statistical treatment of these data was shown. Short et al. did not consider the changes a specific, thiram-induced teratogenic effect. The NOAELs for maternal and developmental toxicity were 100 and 300 mg/kg bw/day, respectively (Sho76).

In an unpublished study, groups of 15 to 20 pregnant New Zealand white rabbits received oral (gavage) doses of thiram (purity: 99.5%) of 0, 1.0, 2.5, or 5.0 mg/kg bw/day on gestational days 6 to 19. The animals were killed on day 29 of gestation. No compound-related mortality was recorded. Maternal body weight gain was decreased in high-dose females. Reproductive parameters, i.e., number of implantation sites, number of resorption sites, number and distribution of live and dead fetuses, were unaffected by treatment, as well as growth and morphological development. The NOAELs for maternal and developmental toxicity were 2.5 and 5 mg/kg bw, respectively (Tes88b).

In another unpublished study, thiram (purity: not given) was administered to groups of 20 pregnant rabbits at doses of 0, 1.0, 5.0, and 10 mg/kg bw on gestational days 7 to 19. The animals were sacrificed on day 29 of gestation. No compound-related mortality or clinical signs of toxicity were seen. Treatment did not induce maternal effects. No adverse effects on fetal development were observed at the teratological examination. The NOAELs for maternal and developmental toxicity were 10 mg/kg bw (Yor92).

In summary, thiram is able to induce delayed ovulation in female rats and sperm abnormalities and testicular necrosis in male rats. The lowest NOAEL for reproductive toxicity in the rat was 2.9 mg/kg bw/day. Mice were much less sensitive to developmental toxicity than rats or rabbits. The lowest NOAEL for developmental effects in the latter species was 5.0 mg/kg bw. Effects on reproduction and development were noted at dose levels which caused maternal toxicity.

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for thiram in the Netherlands is 5 mg/m^3, 8-hour TWA.

Existing occupational exposure limits in some European countries and in the USA are summarised in Annex II.

8 Assessment of health hazard

Workers can be exposed to thiram through inhalation of dust or aerosols or by direct skin contact with the compound or a formulation. The committee did not find data on the respiratory or dermal absorption of the chemical. The extent of absorption following oral administration to rats ranged from 80 to 95%. Following absorption, the majority of the dose was eliminated in the urine

(25-43%) and exhaled breath (41-61%), most of it within the first 12 hours after dosing. About 2-6% of the dose is retained in the carcass at 72 hours after dosing, most of it in the liver, the kidneys, and the blood. There is no evidence of cumulation of the compound in any of these tissues. Thiram is biotransformed into dimethyldithiocarbamate, which is further metabolised into carbon disulphide and a range of oxidative and conjugative polar products. According to the committee, the toxicity of thiram can most probably be attributed to the parent compound and the metabolites carbon disulphide and dimethyldithiocarbamate.

Case studies in humans demonstrated thiram-induced allergic contact dermatitis in workers in the rubber industry, and more recently, in workers who had worn rubber gloves containing thiram and in haemodialysed patients. In factory workers, skin and eye irritation and thyroid disorders have been reported. Reproductive effects have been reported in female operators, allegedly due to exposure to thiram. Thiram induced alcohol intolerance, but to a lesser extent than disulfiram, the ethyl analogue of thiram.

In experimental animals, the compound is slightly irritating to the eyes and the skin, but not a skin sensitiser. Based on the results of acute lethal toxicity studies in test animals, the committee considers the compound as toxic via the inhalation route. When applied via the dermal or oral route, the acute toxicity is of a low order, although large variations were seen between species, strains, and sexes.

Effects of thiram observed in short-term or long-term oral toxicity studies in rats, dogs, and mice included: reduced body weight, anaemia, and degenerative changes of the sciatic nerve in a 2-year study in rats (NOAEL: 1.2 mg/kg bw/day), reduced body weight in a 97-week study in mice, and anaemia and liver and kidney injury in a 104-week study in dogs (NOAEL: 0.4 mg/kg bw/day).

In vitro mutagenicity/genotoxicity tests showed both positive and negative results for gene mutations in bacteria or cultured mammalian cells as well as for cytogenetic effects in cultured mammalian cells. DNA damage (single strand DNA breaks) was demonstrated in cultured testicular cells and peripheral lymphocytes. *In vivo*, 8-week oral administration of thiram at 150 mg/kg bw resulted in dominant lethal mutations in mice, but no increase in the frequency of micronuclei in bone marrow cells of mice was observed at the maximum tolerated dose of 300 mg/kg bw for 8 to 12 days. The committee considers thiram to be mutagenic/genotoxic *in vitro*. *In vivo*, clastogenic or DNA damaging effects were only observed at levels close to the maximum tolerated dose. Carcinogenicity studies in rats and mice did not show treatment-related

increased incidences of neoplastic lesions. The committee concluded that thiram was not carcinogenic in these species.

Effects on the male reproductive system included testicular injury and inactive sperm cells in a 1-year oral toxicity study in rats (NOAEL: 5 mg/kg bw/day). The LOAEL was 5 mg/kg bw/day, based on reduced body weight and changes in activity of testicular enzymes. Thiram induced a delay of the dioestrous phase of the oestrus cycle in rats at a dose of 96 mg/kg bw/day for 14 days. It was demonstrated that an acute dose of thiram is able to block the pre-ovulatory surge of luteinising hormone and to inhibit subsequent ovulation (NOAEL: 12 mg/kg bw). In a 2-generation reproduction toxicity study in rats, the parental and reproductive NOAELs were 1.5 and 2.9 mg/kg bw/day, respectively. In developmental toxicity studies in rats and mice, embryotoxicity was only demonstrated at dose levels which caused maternal toxicity. A recent study in rats showed a NOAEL for developmental toxicity of 7.5 mg/kg bw/day and a LOAEL of 7.5 mg/kg bw for maternal toxicity. Teratogenicity was not observed. In rabbits, NOAELs of 5.0 and 2.5 mg/kg bw/day were found for developmental and maternal toxicity, respectively.

The committee takes the 2-year oral dog study with a NOAEL of 0.4 mg/kg bw as starting point in establishing a health based recommended occupational exposure limit (HBROEL). Since workers are exposed for 5 days a week, this NOAEL from a continuous study (i.e., 7 days a week) is adjusted by multiplying with a factor of 7/5 resulting in a no-adverse-effect level (NAEL) of 0.56 mg/kg bw. For the extrapolation to a HBROEL, a factor of 1.4 for allometric scaling from dogs to humans, based on caloric demand, and an overall factor of 9, covering inter- and intraspecies variation, are applied, resulting in a NAEL for humans of 0.04 mg/kg bw/day. Assuming a 70-kg worker inhales 10 m^3 of air during an 8-hour working day and a retention of 100%, and applying the preferred value approach, a HBROEL of 0.2 mg/m^3 is recommended for thiram. The committee considers a ‘skin notation’ not necessary.

The committee recommends a health-based occupational exposure limit for thiram of 0.2 mg/m^3 as inhalable dust, as an 8-hour time-weighted average (TWA).

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Annex I

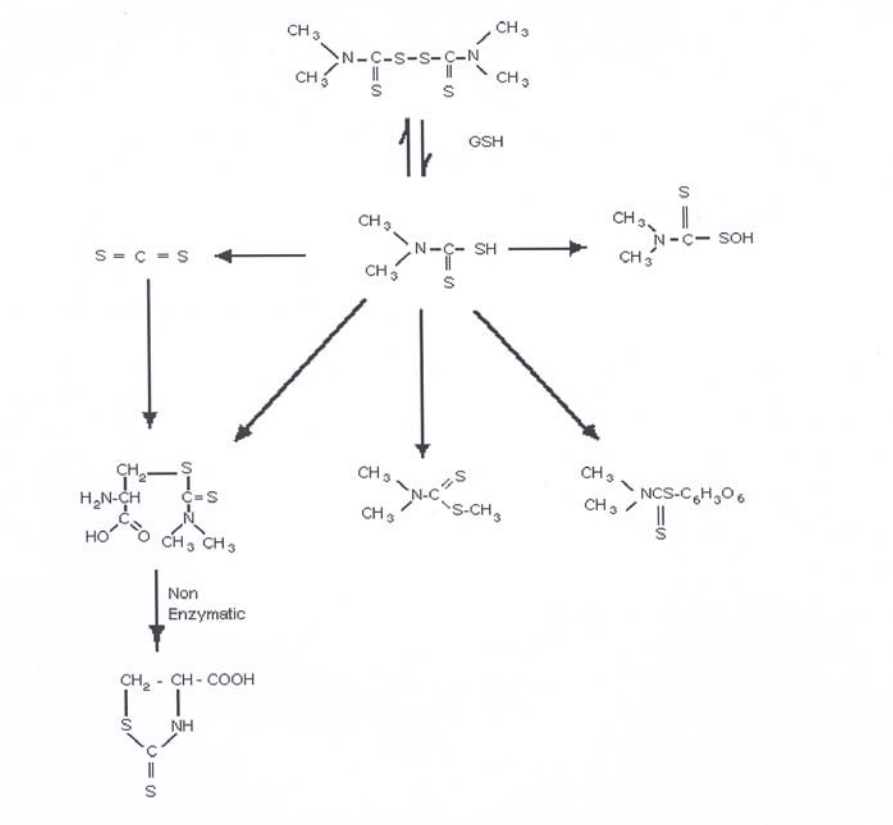

Figure 1 Metabolism of thiram in rats (from FAO93).

Annex II

Occupational exposure limits for thiram in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	-	5	8 h	administrative		SZW03
Germany						
- AGS	-	5[c]	8 h			TRG00
	-	20[c]	15 min			
- DFG MAK-Kommission	-	5[c]	8 h		sens, [e], [f]	DFG02
	-	5[c]	15 min[d]			
Great Britain						
- HSE	-	5	8 h	OES		HSE02
	-	10	15 min			
Sweden	-	-				Swe00
Denmark	-	1	8 h			Arb02
USA						
- ACGIH	-	1	8 h	TLV	A4[g]	ACG03b
- OSHA	-	5	8 h	PEL		ACG03a
- NIOSH	-	5	8 h	REL		ACG03a
European Union						
- SCOEL	-	-				EC03

[a] S = skin notation, which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Measured as inhalable fraction of the aerosol.

[d] Maximum number per shift: 4, with a minimum interval between peaks of 1 hour.

[e] Classified in pregnancy categorie D, i.e., classification in one of the groups A-C is not yet possible because, although the data available may indicate a trend, they are not sufficient for a final classification.

[f] Classified in carcinogen category A4, i.e., not classifiable as a human carcinogen: agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of lack of data. *In vitro* or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.