THIABENDAZOLE (addendum)

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Explanation

Thiabendazole, the International Organization of Standardization (ISO) approved name for 2-(4-thiazolyl)-1*H*-benzimidazole (CAS No. 148-79-8), is a benzimidazole compound used as a systemic fungicide in agriculture. Thiabendazole is also used as a broad-spectrum anthelmintic in various animal species, for control of parasitic infestations in humans, and in materials protection (as a preservative in adhesives, coatings, paper, textiles and paints).

The toxicology of thiabendazole was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1970 and 1977. In 1977, the Meeting established an acceptable daily intake (ADI) of 0–0.3 mg/kg bw on the basis of the absence of effects at 3 mg/kg bw per day in a 6-month study in human volunteers, and a safety factor of 10.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the toxicology of thiabendazole in 1992, 1997 and 2002. In 1997, the Committee established an ADI of 0–0.1 mg/kg bw. In 2002, the Committee established a conservative acute reference dose (ARfD) of 0.1 mg/kg bw based on no-observed-adverse-effect levels (NOAELs) for haemolytic anaemia in repeat-dose studies of toxicity in rats and dogs, in view of the lack of more appropriate acute toxicity data.

After the JECFA evaluation of thiabendazole in 2002, the sponsor conducted several studies of acute toxicity with thiabendazole administered by gavage or in the diet that were designed specifically to derive an ARfD and to determine the pharmacokinetic events that apply under these exposure scenarios. At the request of the Codex Committee on Pesticide residues (CCPR) at its Thirty-eighth Session (Codex Alimentarius Commission, 2006), thiabendazole was re-evaluated at the present Meeting in order to establish an ARfD. The Meeting also reviewed some relevant data from previous evaluations (studies of reproductive toxicity and developmental toxicity, as well as data from humans).

All new studies with thiabendazole were certified as complying with good laboratory practice (GLP).

Evaluation for acute reference dose

1. Biochemical aspects: comparative pharmacokinetics

To better understand the neuroactive effects observed in the studies of toxicity with single doses, several experiments were conducted to relate the observed biological activity of thiabendazole to the pharmacokinetic parameters observed after dosing by gavage or in the diet. This study was evaluated for the first time by the present Meeting.

This study investigated the comparative bioavailability of thiabendazole after a single dose of [^{14}C]thiabendazole (radiochemical purity, > 95%; specific activity, 2.02 MBq/mg) or the equivalent dietary dose over 24 h to CD®:Sprague-Dawley rats. In the gavage experiments, a single dose at 20 or 50 mg/kg bw was administered as a suspension in 0.5% w/v aqueous carboxymethylcellulose to fasted (50 mg/kg bw only) and non-fasted rats. In a separate group whose treatment was designed to mimic the first mouthful of food for the purposes of ARfD determination, a single dose of an aqueous slurry of diet containing [^{14}C]thiabendazole at a concentration of 500 ppm (equal to a mean achieved dose of 0.62 mg/kg bw in males and 0.61 mg/kg bw in females)was given by gavage to non-fasted rats; these rats were then given access to diet containing unlabelled thiabendazole (purity, 99.5%) at a concentration of 500 ppm for 24 h, followed by control diet. Rats in the dietary-kinetics treatment group were given access to diet containing [^{14}C]thiabendazole at a nominal concentration of 200 or 500 ppm, equal to mean achieved doses of 15 mg/kg bw (males and females) and 49 mg/kg bw (males) or 47 mg/kg bw (females) at the lower and higher doses, respectively) for 24 h. In all groups, bioavailability was determined by analysis of one blood sample taken at different times after dosing. There are no specific guidelines for studies of this type, but the study was conducted in accordance with the principles of OECD TG 417 (1984) and was considered to be acceptable.

No adverse clinical signs were recorded in any of the treatment groups. The peak concentration of radioactivity (C_{max}), time of peak concentration (T_{max}), area under the curve (AUC) and half-life in whole blood and in plasma are reported in Table 1 and 2, respectively.

The comparative blood profiles for rats given a single oral dose at 50 mg/kg bw by gavage showed that the presence of diet influenced the rate and extent of absorption of thiabendazole from an aqueous suspension dose. Irrespective of the dietary status of rats, absorption of thiabendazole was rapid. However, in non-fasted rats absorption was more protracted and plasma C_{max} values were lower than in fasted animals. The blood [^{14}C] profiles did not show any marked difference between the sexes. The apparently slow terminal rate of elimination of radioactivity from blood was shown to be attributable to the binding of radioactivity to erythrocytes. The AUC values were greater in the fasted than in the non-fasted animals, which is consistent with the peak concentrations seen in these groups. The values for males were also noticeably greater than for females.

Feeding rats with diets containing thiabendazole at a concentration of 500 ppm confirmed the continued absorption of thiabendazole from diet, with blood concentrations of radioactivity rising over at least 12 h after the presentation of diet, followed by fairly constant blood concentrations until the removal of diet, after which blood radioactivity concentrations began to decline. The slow terminal elimination was again associated with the binding of radioactivity to erythrocytes.

The C_{max} for blood concentrations of radioactivity after exposure to radiolabelled thiabendazole at a dose of 50 mg/kg bw by gavage was about twice the value after dietary exposure for 24 h at 500 ppm (total dose of 49 mg/kg bw for males and 47 mg/kg bw for females). However, the half-lives after exposure by either regimen did not greatly differ.

Table 1. Blood concentrations of radioactivity in rats given a single dose of [^{14}C]thiabendazole by gavage or in the diet

Dose/dietary concentration	Treatment	Sex	Pharmacokinetic parameter			
			C_{max} (µg equivalents/g)	T_{max} (h)	AUC (µg equivalents per h/g)	Half-life (h)
50 mg/kg bw	Non-fasted	Male	13.49	5.05	638.2	68.01
		Female	11.69	4.36	464.5	74.24
	Fasted	Male	21.93	4.15	898.9	132.07
		Female	23.85	2.11	705.9	84.38
500 ppm	Dietary slurry	Male	0.123	0.58	1.363	65.94
		Female	0.102	0.67	1.195	56.75
	Dietary	Male	6.911	13.17	364.0	91.64
		Female	5.841	12.22	267.1	72.33
20 mg/kg bw	Non-fasted	Male	9.47	0.510	178.9	42.37
		Female	11.45	1.010	167.6	34.42
200 ppm	Dietary	Male	1.70	11.98	48.70	45.91
		Female	1.42	12.05	42.28	29.13

From Duerden & Jones (2005)
AUC, area under the curve; C_{max}, peak concentration, T_{max}, time of peak concentration.

Results at the lower doses of 20 mg/kg bw by gavage and 200 ppm in the diet (administered over 24 h, delivering a total dose of 15 mg/kg bw) were compared to determine the linearity of the respective pharmacokinetic parameters. Dosing by gavage again resulted in very rapid absorption, with the C_{max} for radioactivity being attained at approximately 1 h with the characteristic rapid distribution half-life, followed by a much slower terminal half-life elimination. In contrast, after an equivalent dietary dose of 200 ppm, radioactivity was steadily absorbed throughout the exposure interval, with a much lower C_{max} value (about tenfold lower). Again, there was no marked sex difference in either blood or plasma profiles. The comparative blood and plasma curves confirmed the binding of radioactivity to blood cells and also clearly showed that plasma concentrations declined to very low values before the termination of these experiments (at 24 h and 30 h for the groups treated by gavage and with dietary doses, respectively).

Table 2. Plasma concentrations of radioactivity in rats given a single dose of [^{14}C]thiabendazole by gavage or in the diet

Dose	Treatment	Sex	Pharmacokinetic parameter			
			C_{max} (µg equivalents/g)	T_{max} (h)	AUC (µg equivalents per h/g)	Half-life (h)
20 mg/kg bw	Non-fasted	Male	10.17	0.510	46.54	7.37
		Female	12.52	1.010	55.91	8.35
200 ppm	Dietary	Male	1.14	11.98	21.51	9.49
		Female	1.28	12.05	23.21	10.07

From Duerden & Jones (2005)
AUC, area under the curve; C_{max}, peak concentration, T_{max}, time of peak concentration.

In a third set of pharmacokinetic experiments, rats received [^{14}C]thiabendazole at a dose of 500 ppm as an aqueous slurry with diet. The remainder of the daily dietary intake was satisfied by the consumption of diet treated with unlabelled thiabendazole at the same concentration. In essence, this experimental design allowed the fate of the first mouthful of treated diet to be monitored. The

use of this dose form was considered to parallel the creation of such a slurry in the stomach when a rat consumes treated diet and water. The design of this experiment provided an opportunity to follow the absorption of a radiolabelled dietary dose. The requirement for a 10% suspension of diet in water restricted the amount of diet that could be administered. Based upon values for dietary consumption , these rats received an approximate dose of radiolabelled plus unlabelled thiabendazole of 50 mg/kg bw over 24 h. Therefore, this dose was similar to that in rats dosed either by gavage or in dry diet. However, the amount of [^{14}C]thiabendazole administered in the slurry dose was more than 100-fold less than the dose of thiabendazole at 50 mg/kg administered by gavage in 0.5% carboxymethylcellulose. The blood [^{14}C] profiles showed very rapid absorption of radioactivity from thiabendazole with a C_{max} of less than 1 h, followed by a very rapid decline in radioactivity within 2 h after administration, and then by a slower terminal rate of elimination, consistent with observations in the previous groups. Similarly, there was no marked difference between the sexes. These findings confirmed that thiabendazole was rapidly absorbed from the diet. The AUCs for the animals receiving a dose of 500 ppm as dietary slurry and the animals receiving a dietary concentration of 500 ppm were shown to be directly comparable when adjusted for dose. Hence, the fate of the "first mouthful" of radiolabelled diet was indicative of the fate of subsequent dietary intake. The rate of absorption from diet is therefore fast, although not as fast as from a dose in the absence of diet.

In conclusion, an aqueous suspension of thiabendazole was absorbed rapidly when given to fasted and non-fasted rats by oral gavage. However, the extent of absorption was smaller and continued throughout the dietary exposure interval when thiabendazole was administered with diet. At each dose, the comparative gavage and dietary doses were similar (the achieved dose administered in the group receiving a dietary dose at 200 ppm was slightly lower than expected and was attributed to a slightly lower consumption of diet by this group), thereby enabling a direct comparison of blood and plasma radioactivity profiles. C_{max} concentrations of total radioactivity were consistently higher after a gavage dose of an aqueous suspension of thiabendazole than an equivalent dietary dose, indicating that certain effects could be greater if toxicity was determined solely by magnitude of C_{max}. Similarly, AUC values were higher after a gavage dose of a suspension of thiabendazole in carboxymethylcellulose than from a dietary dose. In neither case was there any pronounced difference between the sexes, although AUCs were all higher (7–37% higher depending on the experimental group) in males than in females. After a dose of 50 or 20 mg/kg bw given by gavage to non-fasted rats, the AUC values were consistent with the dose differential. However, in the dietary groups the comparative AUC values were much lower in the group at 200 ppm than in the group at 500 ppm, which was also consistent with the peak concentrations. The half-lives for radioactivity after exposure by either regimen did not differ greatly, indicating that dosing by gavage or in the diet are equally appropriate mechanisms to maintain steady systemic levels of thiabendazole for a sufficient length of time to study its acute hazard potential.

Dietary or gavage studies of acute toxicity (with supporting kinetics) can be equally acceptable for the estimation of hazard potential. However, kinetic studies with dietary slurry provide the best estimate or scenario regarding physiological disposition of compounds after the first mouthful of food containing a high concentration of residue. The administration by gavage of single, high doses of a compound can never mimic human dietary consumption patterns. Nevertheless, this kinetic investigation was conducted to determine the parameters following a plausible, albeit unlikely, high residue exposure scenario (Duerden & Jones, 2005).

2. Toxicological studies

2.1 Studies of toxicity with single doses

These studies were evaluated for the first time by the present Meeting.

In a dose range-finding study, groups of three female CD®:Sprague-Dawley rats were given thiabendazole (purity, 99.4%) at a dose of 0, 100 or 1000 mg/kg bw by gavage on a single occasion, using 0.5% w/v aqueous carboxymethylcellulose as the vehicle. The animals in each group were maintained for 14 days to assess the development of any findings. Clinical observations were recorded at 0.5, 1, 2, 3, 4, 5, 6 and 24 h after dosing and daily thereafter. Body weights and food consumption were measured throughout the study. On day 15, the animals were killed and examined post mortem.

Almost immediately (1–4 h) after dosing at 1000 mg/kg bw by gavage, some clinical signs (sedation, slight piloerection and slight upward curvature of the spine) were observed. Recovery from these initial clinical signs began from 5 h after dosing, with full recovery by 24 h. Although full recovery from the initial clinical effects was apparent, signs indicative of mild systemic toxicity were later seen between days 3 and 8 of the study. Thus, slightly pinched-in sides and upward curvature of the spine were seen again from days 3 to 5, with slight upward curvature of the spine persisting until day 8. Full recovery from these clinical signs was noted by day 9. There were no adverse clinical signs for animals at 100 mg/kg bw. Body-weight loss and reduced body-weight gain were observed between days 1 and 8 for animals (two out of three) at 1000 mg/kg bw. A full recovery in body weight was then seen with these animals overall gaining slightly more weight than the controls. Overall, body-weight gain from days 1 to 15 at 1000 mg/kg bw was similar to controls. There were no effects of treatment on the body weights of animals at 100 mg/kg bw. There was an associated reduction in food consumption among animals at 1000 mg/kg bw on days 1 to 4, with consumption similar to, or slightly higher than, that of controls for the remainder of the study. A slight reduction in food consumption was also present, on day 1 only, for animals at 100 mg/kg bw. However, in the absence of any associated effects on body weight, this was considered not to be of toxicological significance. No abnormalities were observed upon examination post mortem.

A single dose of 1000 mg/kg bw was associated with clinical signs and reduced body weight indicative of mild systemic toxicity, and sedation indicative of a possible neuroactive effect. The time to peak effect for the clinical observations was between 3 and 4 h after dosing. Full recovery from these effects was observed by day 9 and day 15, respectively. The NOAEL was 100 mg/kg bw. Accordingly, the doses chosen for the first study ranged between 100 and 1000 mg/kg bw (Noakes, 2004a).

In the first study, groups of 10 male and 10 female non-fasted CD®:Sprague-Dawley rats were given thiabendazole (purity, 99.4%) at a dose of 0, 100, 200 or 1000 mg/kg bw by gavage on a single occasion using 0.5% w/v aqueous carboxymethylcellulose as the vehicle. Half of the animals in each group were killed after 24 h and the remainder were maintained on study for up to 14 days to assess the development of any findings. Clinical observations were recorded at 0.5, 1, 2, 4 and 24 h after dosing and daily thereafter. Body weights and food consumption were measured throughout the study. In addition, detailed clinical observations, including a qualitative assessment of sensory perception and quantitative assessments of landing-foot splay and muscle weakness, and assessment of motor activity were performed 3 h after dosing, 24 h after dosing and on day 15. Urine samples for clinical chemistry were collected on days 2 and 14. After either 24 h or 14 days, the animals were killed and examined post mortem. Sample of cardiac blood were taken for clinical pathology, selected organs (adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus and uterus with cervix) were weighed and specified tissues were taken for subsequent histopathological examination. The study was performed according to the proposed test guideline document published by JMPR 2000, *Proposed test guideline – single-dose toxicity study by the oral route (for use in establishing acute reference doses for chemical residues in food and drinking water)*[1] .

[1] The document entitled *Guidance document for setting an acute reference dose (ARfD) (prepared by Germany)* from the European Commission, Directorate General for Agriculture VI B II.1, 7199/VI/99 rev. 3, dated 2 August 1999, was also consulted.

At 1000 mg/kg bw, qualitative effects observed in all the test animals included slightly decreased activity in all males at 1 h and in all females at between 0.5–2 h after dosing. Tiptoe gait was observed at 4 h in 1 of 10 males. Full recovery from these effects occurred by 24 h after dosing, except for one male who showed full recovery on day 3. At 100 and 200 mg/kg bw, slightly decreased activity was observed for males and females between 0.5 or 1 and 4 h after dosing on day 1. Tiptoe gait was observed in 2 of 10 males at 200 mg/kg bw between 2 and 4 h after dosing. No adverse clinical signs were present by 24 h after dosing. Compared with controls, body weights were decreased for males and females at 1000 mg/kg bw; the maximum differences occurred on days 4–5 and were 11% and 7% and for males and females, respectively. Full recovery for females and a partial recovery for males were seen by day 15. Correspondingly, food consumption was slightly lower on days 1–5 for males and females, relative to controls. At 200 mg/kg bw, body weights were marginally lower, by about 3%, in males on day 2 only.

During the detailed functional observation battery (FOB), slight tiptoe gait was observed in several treated animals at 3 h after dosing (Table 3).

Slightly decreased activity was also recorded and a slightly reduced righting reflex was seen at 3 h in some animals at 200 or 1000 mg/kg bw. At 24 h after dosing, slightly decreased activity was still present in few males at 1000 mg/kg bw. None of these effects were present on day 15 of the study. Landing-foot splay was reduced for females only at 3 h after dosing and for males and females at 24 h after dosing. By day 15, landing-foot splay values were similar to controls. Motor activity was reduced for males and females treated with 1000 mg/kg bw at 3 h and 24 h after dosing, but was similar to the controls by day 15. At 100 and 200 mg/kg bw, motor activity was reduced for males only, at 3 h after dosing.

Table 3. Results of a detailed functional observation battery (No. of animals affected) in a study of acute toxicity in rats given thiabendazole by gavage

Parameter	Dose (mg/kg bw)							
	Males				Females			
	0	100	200	1000	0	100	200	1000
1–4 h								
Tip to gait	0	3	4	2	0	3	3	3
Decreased activity	0	0	4	8	0	0	0	2
Reduced splay reflex	0	0	0	0	0	0	0	0
Reduced righting reflex	0	0	2	1	0	0	0	0
24 h								
Tip to gait	0	0	0	0	0	0	0	0
Decreased activity	0	0	0	3	0	0	0	0
Reduced splay reflex	0	0	0	0	0	0	0	0
Reduced righting reflex	0	0	0	0	0	0	0	0
Day 15								
Tip to gait	0	0	0	0	0	0	0	0
Decreased activity	0	0	0	0	0	0	0	0
Reduced splay reflex	0	0	0	0	0	0	1	0
Reduced righting reflex	0	0	0	0	0	0	0	0

From Noakes (2004b)

There were no treatment-related changes in haematology parameters measured on days 2 and 14. At 1000 mg/kg bw, and only on day 2, cholesterol and triglyceride concentrations were slightly

higher for males (125% and 172% of that of controls, respectively) and females (125% and 145% of that of controls, respectively), urea concentration was lower for males and females (80% and 67%), alanine aminotransferase activities (73%) and plasma calcium concentration (96%) were slightly lower for males, total plasma protein was slightly higher for males (107%), glucose and phosphorus concentrations were slightly lower for females (73% and 87%, respectively). Urinary pH was slightly higher for males on day 2. No treatment-related blood or urine clinical chemistry changes were observed on days 15 or 14 of the study, respectively. At 200 mg/kg bw, cholesterol concentrations were slightly higher for females (126% of control) and alanine aminotransferase activities (69%) and plasma calcium (96%) were slightly lower for males on day 2 only. There were no effects at 100 mg/kg bw.

Relative liver weights at 1000 mg/kg bw were slightly higher for males and females on day 2 only (111% and 110% of control, respectively). Absolute and relative brain (105% and 106% of control, respectively) and heart weights (118% of control for both) were slightly higher for females on day 15. There were no histopathological findings associated with treatment.

Dose-related effects involving reduced activity, tiptoe gait, landing-foot splay and reduced motor activity were observed in males and females up to 24 h after dosing at 100 or 200 mg/kg bw or up to 3 days after dosing at 1000 mg/kg bw. At the highest dose, there was also a reduction in body weight compared with controls. Recovery from these effects was observed by day 15. The minor reversible blood chemical chemistry changes, in the absence of any treatment-related histopathological findings, were considered not to be of toxicological importance. Similarly, the slight and transient liver-weight effect and the slight brain- and heart-weight effects, observed for females only, in the absence of any histopathological changes in these organs, were considered not to be of toxicological significance. A clear NOAEL was not identified in this study (Noakes, 2004b).

In order to better understand the nature and duration of the reversible neuroactive effects observed in the main single-dose study of toxicity (Noakes, 2004b), and also to establish clear NOAELs for all observation periods, an additional study was conducted. In this second study, groups of 10 male and 10 female non-fasted CD®:Sprague Dawley rats were given thiabendazole (purity, 99.4%) at a dose of 0, 20, 50 or 100 mg/kg bw by gavage on a single occasion using 0.5% w/v aqueous carboxymethylcellulose as the vehicle. Half of the animals in each group were killed after 24 h and the remainder were maintained for up to 14 days to assess the development of any findings. Clinical observations were recorded at 0.5, 1, 2, 4 and 24 h after dosing and daily thereafter. Body weights and food consumption were measured throughout the study. In addition, detailed clinical observations, including a qualitative assessment of sensory perception and quantitative assessments of landing-foot splay and muscle weakness, and assessment of motor activity were performed 3 h after dosing, 24 h after dosing and on day 15. Since haematological parameters were not affected in the main study and clinical chemistry parameters were not considered to be affected in any toxicologically significant way (Noakes, 2004b), appropriate samples, including cardiac blood, were collected and stored, but were not analysed in this study. After either 24 h or 14 days, the animals were killed and examined post mortem. Selected organs were weighed and specified tissues taken and stored; however, histopathological examinations were not conducted. The study was performed according to the proposed test guideline document published by JMPR 2000: *Proposed test guideline – single-dose toxicity study by the oral route (for use in establishing acute reference doses for chemical residues in food and drinking water)*[2].

[2] The document entitled *Guidance document for setting an acute reference dose (ARfD) (prepared by Germany)* from the European Commission, Directorate General for Agriculture VI B II.1, 7199/VI/99 rev. 3, dated 2 August 1999, was also consulted.

At 100 mg/kg bw, slightly decreased/subdued activity was seen during qualitative clinical observations in up to two males and two females on days 1–2, and tiptoe gait was observed in up to two males and two females from 2–48 h after dosing. A reduced splay reflex was seen in two females on day 2. Full recovery in all of these parameters occurred by 48 h after dosing.

At 50 mg/kg bw, qualitative clinical observations show slightly decreased/subdued activity in three males and two females on day 2, and tiptoe gait in two males and two females from 4 h after dosing. Reduced splay reflex was seen for one female on day 2 after treatment. On days 3 and 4, decreased activity was seen in one male only. Full recovery in all clinical parameters was apparent by 4 days after dosing.

At 20 mg/kg bw, slightly decreased activity was seen during clinical observation of one male from 0.5 to 1 h after dosing, and for three males and two females on day 2, and tiptoe gait was observed for two males and two females on day 2. A reduced splay reflex was seen for one female on day 2 of treatment. Full recovery of all signs was apparent by 2 days after dosing.

These reversible neuroactive effects were similar in severity and duration to those observed at 100 mg/kg in the preceding study in rats treated by gavage (Noakes, 2004b).

There were no toxicologically significant effects on body weight. Slightly low food consumption was seen for females treated with thiabendazole at 100 mg/kg bw on day 1 only.

Table 4. Results of a detailed functional observation battery (No. of animals affected) in a second study of acute toxicity in rats given thiabendazole by gavage

Parameters	Dose (mg/kg bw)							
	Males				Females			
	0	20	50	100	0	20	50	100
1–24 h								
Decreased activity	0	3	2	1	0	0	1	3
Reduced splay reflex	0	0	1	0	0	0	1	1
Reduced righting reflex	0	0	0	0	0	0	0	0
1-2 days								
Decreased activity	0	3	3	2	0	2	2	0
Reduced splay reflex	0	0	0	0	0	1	1	2
Reduced righting reflex	0	0	0	0	0	0	0	0
Day 15								
Decreased activity	0	0	0	0	0	0	0	0
Reduced splay reflex	0	0	0	0	0	0	1	0
Reduced righting reflex	0	0	1	0	0	0	0	0

From Noakes (2005a)

During the detailed FOB assessments, slightly decreased activity was observed 3 h after dosing for one male and three females at 100 mg/kg bw, two males and one female at 50 mg/kg bw, and three males at 20 mg/kg bw (Table 4).

A slightly reduced foot-withdrawal reflex was seen for one of 10 males from each of the groups at 20, 50 or 100 mg/kg bw and one of 10 females at 100 mg/kg bw. A slightly reduced splay reflex was also seen in one female at 100 mg/kg bw and in one male and one female at 50 mg/kg bw. Slightly decreased activity was still present 24 h after dosing for two males at 100 mg/kg bw, three males and two females at 50 mg/kg bw and three males and two females at 20 mg/kg bw. In contrast with the previous study (Noakes, 2004b), landing-foot splay was unaffected by treatment at any time. Overall motor activity was reduced for males at 100 mg/kg bw, 3 h after dosing. Slightly reduced motor

activity was most apparent for this group at 6–20 min and 26–30 min. There were no statistically significant differences in motor activity, in either sex, 24 h after dosing. No treatment-related adverse effects were present on day 15.

No treatment-related macroscopic abnormalities were observed during post-mortem examinations carried out at 24 h or 14 days.

In this repeat single-dose study of toxicity in rats treated by gavage, some minor and reversible clinical signs affecting motion characteristics were observed in all groups treated with thiabendazole, but generally at a low severity and incidence. Taking into account the overall response across this study and the preceding study, there was no obvious dose–response relationship in terms of numbers of animals affected or the severity of the findings. There did appear to be an effect of treatment on motor activity for males at 100 mg/kg bw, but this was only present at 3 h after dosing and the difference to controls was only relatively small. All these putative neuroactive effects were clearly reversible, with full recovery apparent by 14 days after dosing. In this study, there were no quantifiable differences in landing-foot splay for all groups of animals that received thiabendazole. The NOAEL was 100 mg/kg bw (Noakes, 2005a).

As an extension of these studies of toxicity with single doses administered by gavage and to confirm the NOAEL identified for the dietary route, groups of 10 male and 10 female CD®:Sprague-Dawley rats were fed diets containing thiabendazole (purity, 99.4%) at a concentration of 0, 300, 400, or 600 ppm for approximately 24 h. Achieved doses were equal to 0, 26, 34 and 48 mg/kg bw for males and 0, 26, 33 and 46 mg/kg bw for females. Feeding of experimental diets commenced immediately before the dark cycle on day –1 until the next dark cycle on day 1. Half the animals in each group were killed after 24 h and the rest were maintained on control diet for 14 days to assess the development of any findings. Clinical observations were recorded before feeding (day –1) then on day 1 at approximately 0.5 h (as part of an FOB), 2, 4 and 24 h (as part of an FOB) after the dark cycle ended on day 1, and at least once each day from days 3 to 14 (at approximately the same time) and on day 15 (as part of FOB). Body weights and food consumption were measured throughout the study. In addition, detailed clinical observations, including a qualitative assessment of sensory perception and quantitative assessments of landing foot splay, and assessment of motor activity were performed as soon as possible after the end of the dark cycle on day 1, to coincide with the time of peak effect of signs that could be related to peak plasma concentrations (T_{max}) measured in the study of kinetics after dietary administration (Duerden & Jones, 2005), 24 h after that (on day 2) and on day 15 before termination. After either 24 h or 14 days, the animals were killed and examined post mortem and specified tissues were taken for possible future histopathology examination. The study was performed according to the proposed test guideline document published by JMPR 2000, *Proposed test guideline – single-dose toxicity study by the oral route (for use in establishing acute reference doses for chemical residues in food and drinking water)*.[3] According to European Union[4] and JMPR guidance criteria, intake of test substance via dietary exposure is an acceptable dosing surrogate for gavage administration for the estimation of ARfD. The same conclusion can also be drawn based on results of comparative studies using administration by gavage or in the diet (Duerden & Jones, 2005).

[3] The document entitled *Guidance document for setting an acute reference dose (ARfD) (prepared by Germany)* from the European Commission, Directorate General for Agriculture VI B II.1, 7199/VI/99 rev. 3, dated 2 August 1999, was also consulted.

[4] *Opinion of the Scientific Committee on Plants on the draft guidance document for the setting of an acute reference dose (ARfD)* (SCP/GUIDE-ARFD/002-Final) from the European Commission Health & Consumer Protection Directorate General, dated 18 July 2002, Scientific Committee on Plants; Available from http://ec.europa.eu/food/fs/sc/scp/out133_ppp_en.pdf.

There were no treatment-related clinical signs at any dose. Reduced splay reflexes were observed among control and treated groups; however, the incidences and severity showed no relationship to treatment with thiabendazole and were considered to be incidental (Table 5).

Table 5. Detailed functional observation battery (No. of animals affected) in a study to establish an acute reference dose in rats given diets containing thiabendazole

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	300	400	600	0	300	400	600
1–24 h								
Decreased activity	0		0	0	0	0	0	0
Reduced splay reflex	0	2	0	1	4	4	2	2
Reduced righting reflex	0	+	0	0	0	0	0	0
1–2 days								
Decreased activity	0	0	0	0	0	0	0	0
Reduced splay reflex	0	3	1	0	2	3	2	3
Reduced righting reflex	0	0	0	0	0	0	0	0
Day 15								
Decreased activity	0	0	0	0	0	0	0	0
Reduced splay reflex	1	2	2	1	1	1	0	1
Reduced righting reflex	+	0	0	0	+	0	0	0

From Noakes (2005b)
+, qualitative effects.

FOB assessments and motor activity measurements did not reveal any treatment-related changes. Body weight and macroscopic findings showed no effects of treatment with thiabendazole. Although there appeared to be a slight reduction in food consumption for both males and females at 600 ppm for the 24 h during which the experimental diet was given; this was not accompanied by any associated reduction in body weight.

Clinical signs, FOB, motor activity and other investigations conducted in this study were initially performed shortly after the end of the dark cycle when plasma concentrations of thiabendazole were known to be at their highest level, based on the results from the preceding study of kinetics (Duerden & Jones, 2005). In view of the absence of treatment-related effects or clinical signs under this experimental scenario, the NOAEL for thiabendazole was at least 600 ppm (equal to 48 mg/kg bw for males and 46 mg/kg bw for females), the highest dose tested (Noakes, 2005b).

2.2 Reproductive toxicity

The following studies have previously been considered by JECFA and were reviewed by the present Meeting.

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 33 male and 33 female Sprague-Dawley Crl:CD(SD)BR rats received diets containing thiabendazole (purity, > 99%) at concentrations providing a dose of 0, 10, 30 or 90 mg/kg bw per day. This study was summarized by JECFA in 1997 (JECFA, 1997). The only treatment-related findings were effects on food

consumption and body-weight gain in parental animals and offsprings, for which JECFA previously identified a NOAEL of 10 mg/kg bw per day. These effects were considered irrelevant for acute exposure. The NOAEL for reproduction was 90 mg/kg bw per day (the highest dose tested) based on a lack of any effect on reproductive performance (Wise & Lankas, 1992).

(b) Developmental toxicity

Mice

Three studies of developmental toxicity were undertaken in pregnant Jcl:ICR mice. Thiabendazole (purity, 98.5%) was given as a suspension in olive oil by gastric intubation. The animals were killed on day 18 of gestation. These studies were summarized by JECFA in 1992 (JECFA, 1993) and were reviewed by the present Meeting.

In the first experiment, mice were given thiabendazole at a dose of 0, 700, 1300 or 2400 mg/kg bw per day on days 7–15 of gestation. All fetuses were removed from the uterus on day 18 of gestation. Maternal body-weight gain was decreased in a dose-related fashion at all doses, and the mortality rate increased with increasing dose, being 0 out of 39 (controls), 0 out of 39 (lowest dose), 5 out of 39 (intermediate dose) and 24 out of 39 (highest dose). The weights of the liver, kidney, heart and spleen were increased at all three doses. A dose-related increase in the frequency of resorptions and a decrease in the number of live fetuses was seen at the two higher doses, while a dose-related decrease in fetal body weight occurred at all doses. In the offspring, the incidence of cleft palate was increased in a dose-related fashion at all doses, and the incidence of fusion of vertebral arches and vertebral bodies was increased in offspring of dams at the two lower doses. At the highest dose, only 20 fetuses from 3 litters were examined for external malformations and 15 fetuses from 2 litters for skeletal malformations (Ogata et al., 1984).

In the second experiment, animals were given thiabendazole at a dose of 2400 mg/kg bw on a single day between days 6 and 15 of gestation. All fetuses were removed from the uterus on day 18 of gestation. The maternal mortality rates were 2/7, 2/12, 1/12, 2/11, 2/11, 6/11, 2/11, 1/11, 4/11 and 6/11 on days 6–15 of gestation, respectively. The number of gestating females was markedly reduced in groups treated with thiabendazole on day 6 or 7 of gestation (1/7, 6/12, 9/12, 7/12, 9/11, 5/11, 9/11, 10/11, 7/11 and 4/11 on days 6–15, respectively). The rate of resorptions was increased and the fetal body weight decreased after dosing on any day. Increased frequencies of microcephaly and exencephaly were seen after treatment on day 6, 7 or 8; short or absent tail and anal atresia after dosing on day 9; open eyelids after treatment on day 7, 8, 10, 13 or 14; reduction deformity of the limbs after dosing on day 9, 10, 11 or 12; cleft palate after dosing on day 8, 9, 10, 11, 12 or 13; fusion of vertebral arches and vertebral bodies after treatment on day 7, 8, 9, 10 or 13; and fusion of the ribs after dosing on day 7, 8 or 9 (Table 6).

The reduction deformity of limbs observed was very significant (Ogata et al., 1984). This anomaly was not found in approximately 6000 fetuses of normal Jcl:ICR mice examined in this laboratory. In other laboratories, the spontaneous occurrence of this anomaly in this strain of mice was very low (0.02% amelia (absence of limb) and 0.02% oligodactilia among 5000 fetuses in one laboratory, and no deformities were seen in 4335 fetuses in another laboratory) (Kameyama, Tanimura & Yasuda, 1980).

Table 6. Incidence of malformations in fetuses of mice given thiabendazole at a dose of 2400 mg/kg bw orally on one of days 6–15 of gestation

Observation	Day of gestation									
	6	7	8	9	10	11	12	13	14	15
External malformations										
No. of litters with malformed fetuses/No. examined	1/1	3/6	2/9	4/7	4/8	3/4	2/7	7/10	1/6	0/2
No. of malformed fetuses/No. examined	1/15	6/64	3/108	11/58	5/92	23/41	3/81	28/125	1/79	0/24
No. of fetuses with:										
Microcephalia	1	0	0	0	0	0	0	0	0	0
Exencephalia	0	3	1	0	0	0	0	0	0	0
Reduction deformity of limbs	0	0	0	10	1	7	1	0	0	0
Short or absent tail	0	0	0	5	0	0	0	0	0	0
Anal atresia	0	0	0	4	0	0	0	00	0	0
Cleft palate	0	0	1	3	4	20	3	22	0	0
Open eyelids	0	5	1	0	2	0	0	6	1	0
Skeletal malformations										
No. of litters with malformed fetuses/No. examined	—	6/6	7/9	4/6	1/8	0/3	0/7	1/10	0/6	0/2
No. of malformed fetuses/No. examined	—	19/64	24/107	16/54	1/92	0/35	0/81	1/125	0/79	0/24
No of fetuses with:										
Fusion of vertebral arches	—	15	10	14	1	0	0	1	0	0
Fusion of vertebral bodies	—	1	1	1	0	0	0	0	0	0
Fusion of ribs	—	3	18	1	0	0	0	0	0	0

From Ogata et al. (1984)

In the third experiment, groups of 21–31 mice were given one of 17 different doses of thiabendazole at between 30 and 2400 mg/kg bw on day 9 of gestation. All fetuses were removed from the uterus on day 18 of gestation. Maternal body-weight gain was decreased at ≥ 1200 mg/kg bw, maternal mortality rate was increased at ≥ 1700 mg/kg bw (2/31, 3/31 and 7/31 females died at 1700, 2000 and 2400 mg/kg bw, respectively), and the weights of the liver, heart and kidney were decreased at ≥ 1400 mg/kg bw. The incidence of resorbed fetuses was increased at 1700 mg/kg bw, the mean number of live fetuses was significantly decreased at ≥ 2000 mg/kg bw and fetal body weight was decreased at ≥ 60 mg/kg bw. The incidences of external malformations such as cleft palate, exencephaly, open eyes lids and omphalocele (outpouching of the umbilicus containing internal organs) were similar in the control and treated groups. The incidence of reduction deformity of the limbs was increased at ≥ 480 mg/kg bw (statistically significantly from at 1200 mg/kg bw), and that of fusion of vertebral arches and vertebral bodies and of ribs was increased at ≥ 240 mg/kg bw (Tables 7 and 8) (Ogata et al., 1984).

Table 7. Incidence of external malformations in fetuses of mice given thiabendazole at a dose of 30–2400 mg/kg bw orally on day 9 of gestation

Dose (mg/kg bw) (grouped by experiment)	No. of litters with malformed fetuses/No. examined[a]	Percentage with malformation[b]	No. of malformed fetuses/No. examined	No. of fetuses with:						
				Reduction deformity of limbs[c]	Short or absent tail	Anal atresia	Cleft palate	Open eyelids	Exencephalia	Omphalocele
2400	9/18 (50.0)	24.6 ± 37.7*	16/179	9 (8; 44.4)***	6*[d]	0	0	0	0	0
2000	9/22 (40.9)	18.6 ± 34.2	14/189	7 (6; 27.3)***	2	0	2	7	2	0
1667	10/27 (37.0)	7.9 ± 13.7	17/305	7 (6; 22.2)**	8	0	0	5	0	0
1389	10/21 (47.6)	18.8 ± 31.1*	34/242	26 (6; 28.6)***	17*[d]	8	8	2	2	1
1127	4/20 (20.0)	6.5 ± 16.6	9/240	6 (3; 15.0)*	4	0	0	1	0	0
0	6/18 (33.3)	4.7 ± 9.7	11/246	0	0	0	2	6	3	0
1157	6/21 (28.6)	8.3 ± 22.9	8/213	2 (2; 9.5)	1	0	2	5	0	0
965	6/21 (28.6)	2.8 ± 4.7	7/251	0	0	0	0	7	1	0
804	4/22 (18.2)	1.9 ± 4.4	5/252	1 (1; 4.6)	0	0	0	4	0	0
670	4/19 (21.1)	3.5 ± 7.2	5/225	1 (1; 5.9)	0	0	0	4	0	0
558	4/22 (18.2)	1.8 ± 4.1	6/277	0	0	0	0	5	1	0
0	4/21 (19.0)	1.5 ± 3.3	4/244	0	0	0	2	1	1	0
558	3/21 (14.3)	1.9 ± 4.8	5/288	1 (1; 4.8)	0	0	0	4	0	0
269	3/23 (13.0)	1.5 ± 4.1	3/295	0	0	0	0	3	0	0
129	4/21 (19.0)	1.3 ± 2.8	4/294	0	0	0	0	3	1	0
62	4/22 (18.2)	2.2 ± 4.9	7/295	0	0	0	0	5	3	0
30	4/22 (18.2)	2.0 ± 4.9	6/300	0	0	0	0	6	0	0
0	3/20 (15.0)	0.7 ± 2.1	3/274	0	0	0	0	1	1	1
480	9/28 (32.1)	3.4 ± 6.3	13/365	2 (2; 7.1)	1	0	0	8	2	0
240	5/25 (20.0)	1.7 ± 3.9	5/362	0	0	0	0	5	0	0
120	6/27 (22.2)	2.3 ± 4.9	7/256	0	0	0	1	6	0	0

Dose (mg/kg bw) (grouped by experiment)	No. of litters with malformed fetuses/No. examined[a]	Percentage with malformation[b]	No. of malformed fetuses/No. examined	No. of fetuses with:						
				Reduction deformity of limbs[c]	Short or absent tail	Anal atresia	Cleft palate	Open eyelids	Exencephalia	Omphalocele
60	3/27 (11.1)	1.8 ± 6.1	5/361	0	0	0	3	2	1	0
30	4/28 (14.3)	1.2 ± 3.1	5/387	0	0	0	0	5	0	0
0	2/27 (7.4)	0.5 ± 1.9	2/347	0	0	0	0	2	0	0

From Ogata et al. (1984)

[a] Shown as a percentage in parentheses.

[b] Calculated by averaging the percentage in each litter (i.e. No. of malformations/No. of fetuses) and shown as mean ± standard deviation.

[c] In parentheses: No. of litters with affected fetuses; percentage of affected litters among those examined.

[d] The chi-squared test was used to compare the number of affected litters.

Values marked with asterisks are significantly different from those in the corresponding control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) as determined by the following statistical tests: chi-squared test (total incidence of litters with malformed fetuses and incidences of litters having fetuses with specific malformations); rank sum test (% incidence of malformations).

The paper by Ogata et al. (1984; see above) states that the time dependency for thiabendazole-induced malformations indicates that the compound is a non-specific teratogen. This time dependency is also characteristic of effects secondary to maternotoxicity. Cleft palate, exencephaly, open eye lids and vertebral anomalies are commonly observed in mice exposed to highly toxic doses of a variety of chemicals (Kavlock et al., 1985; Khera, 1984). Metabolic acidosis induced by carbon dioxide (CO_2) produces reduction limb deformity in mice (Weaver & Scott, 1984). Lack of controls (experiment 2) and precise data on maternal toxicity (only mortality was reported) compromise interpretation of the results of these three experiments. Excessive doses were used resulting in excessive maternal lethality and probably excessive toxicity (see further study in which decreases of 15 and 24% body-weight gain were observed in dams treated with thiabendazole at 100 or 200 mg/kg bw between days 6–15 of gestation). The number of fetuses/litters examined was sometimes very low, especially in the two first experiments. In the first two experiments, the authors reported the number of fetuses with specific malformations but not the number of litters affected. Therefore, the present Meeting considered the results of these three experiments as providing supplementary information only.

In a study of developmental toxicity, groups of 25 pregnant Jcl:ICR mice were given thiabendazole (purity, 99.8%) at a dose of 0, 25, 100 or 200 mg/kg bw per day in olive oil by oral gavage on days 6–15 of gestation. The animals were killed on day 18 of gestation. This study was evaluated by JECFA in 1997 (JECFA, 1997) and re-evaluated by the present Meeting. Dams at the two higher doses showed dose-related decreases in food consumption (13% below control levels on days 12–14 at the intermediate dose and 9–13% below control levels on days 6–8, 12–14, 14–16 and 16–18 at the highest dose) and body-weight gain (15% and 24% at the intermediate and highest dose, respectively), and there were dose-related decreases in the number of implantations (16.1, 15.4, 14.8 and 14.3 implants per pregnant female in the control group and at the lowest, intermediate and highest dose, respectively. The decrease was statistically significant ($p \leq 0.05$) at the two higher doses), dose-related decreases in the number of live fetuses (15.3, 14.6, 14.2 and 13.7, the decrease was statistically significant ($p \leq 0.05$) at the two higher doses) and statistically significant decreases in fetal body weight (97% and 95% of control values for females and 97% and 96% of control values for males at the intermediate and highest dose, respectively) at the two higher doses. An increased incidence of delayed ossification at a single site talus calcaneus was seen at all doses, but this was not dose-related, the number of affected litters was similar in all groups, including controls and was not considered to be a specific effect of thiabendazole on skeletal ossification. The NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of decreases in food consumption and body-weight gain at doses of 100 mg/kg bw per day or greater. The NOAEL for developmental toxicity was 25 mg/kg bw per day on the basis of the reduced number of implantations and live fetuses at doses of 100 mg/kg bw per day or greater (Nakatsuka et al., 1995).

Table 8. Incidence of skeletal malformations in fetuses of mice given thiabendazole at a dose of 30–2400 mg/kg bw orally on day 9 of gestation

Dose (mg/kg bw)	No. of litters with malformed fetuses/No. examined[a]	% malformed[b]	No; of malformed fetuses/No. examined	No. of fetuses with fusion of:		
				Vertebral arches	Vertebral bodies	Ribs
2400	12/18 (66.7)***	44.6 ± 42.3***	64/179	45	19	24
2000	17/21 (81.0)***	41.7 ± 39.0***	55/181	46	18	10
1667	18/27 (66.7)***	25.3 ± 28.2***	70/305	58	15	12
1389	13/21 (61.9)***	31.5 ± 39.1***	61/242	51	29	18
1127	11/19 (57.9)***	13.9 ± 17.9***	26/228	16	9	6
0	0/17 (0)	0	0/232	0	0	0

1157	12/21 (57.1)***	27.2 ± 33.9***	50/213	36	6	17
965	9/21 (42.9)*	11.6 ± 25.0***	27/251	19	9	5
804	9/22 (40.9)*	16.4 ± 23.6***	41/252	23	14	12
670	9/19 (47.4)**	15.2 ± 26.0***	36/225	34	4	5
558	9/22 (40.9)*	11.2 ± 16.9***	30/277	27	4	6
0	1/21 (4.8)	0.4 ± 1.8	1/244	0	1	0
558	7/21 (33.3)	8.1 ± 15.4*	22/288	18	1	5
269	4/23 (17.4)	1.5 ± 3.4	4/295	2	0	2
129	0/21 (0)	0	0/294	0	0	0
62	1/22 (4.5)	0.4 ± 1.6	1/295	1	1	1
30	0/22 (0)	0	0/300	0	0	0
0	1/20 (5.0)	0.4 ± 1.7	1/274	1	0	9
480	16/27 (59.3)***	16.7 ± 27.5***	52/352	46	0	7
240	7/25 (28.0)*	6.4 ± 16.3**	28/362	19	0	16
120	2/27 (7.4)	0.5 ± 2.0	2/256	0	1	1
60	2/27 (7.4)	0.7 ± 2.5	2/361	1	0	1
30	0/28 (0)	0	0/387	0	0	0
0	0/27 (0)	0	0/347	0	0	0

From Ogata et al. (1984)

[a] Shown as a percentage in parentheses.

[b] Calculated by averaging the percentage in each litter (i.e. No. of malformations/No. of fetuses) and shown as mean ± standard deviation.

Values marked with asterisks are significantly different from those in the corresponding control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) as determined by the following statistical tests: chi-squared test (total incidence of litters with malformed fetuses); rank sum test (% incidence of malformations).

Rats

In a study of developmental toxicity, groups of 25 pregnant Sprague-Dawley Crl:CD(SD) BR rats received thiabendazole (purity, 98.9%) at a daily dose of 0, 10, 40 or 80 mg/kg bw in 0.5% methylcellulose by gavage on days 6–17 of gestation. The animals were killed on day 20 of gestation. This study was evaluated by JECFA in 1992 (JECFA, 1993) and re-evaluated by the present Meeting. Food consumption and body-weight gain of the dams at the two higher doses were decreased in a dose-related manner (food consumption, 11–15% at the intermediate dose and 22–28% at the highest dose; body-weight, 2% at the intermediate dose and 26% at the highest dose), and dams at the highest dose showed ptosis (day 6 of gestation) and regurgitation. A dose-related decrease in fetal body weight was seen at the two higher doses (5% and 6% less than control values in females and 3% and 5% in males at the intermediate and highest dose, respectively). The NOAEL for maternal and developmental toxicity was 10 mg/kg bw per day (Wise, 1990; Lankas & Wise, 1993).

Rabbits

In a study of developmental toxicity, groups of 18 pregnant New Zealand White rabbits received thiabendazole (purity, 98.9%) at a dose of 0, 24, 120 or 600 mg/kg bw per day by gavage in 0.5% methylcellulose on days 6–18 of gestation. The animals were killed on day 29 of gestation. This study was evaluated by JECFA in 1992 (JECFA, 1993) and re-evaluated by the present Meeting. Dose-related decreases in food consumption (about 37–38% of control values on days 12–16 and 16–19)

and body-weight gain (decrease of 14% at 120 mg/kg bw per day) were seen at the two higher doses, with a loss of body weight at the highest dose (–230 g during the dosing period). Four out of 18 dams at the highest dose aborted. The mortality rates were 2 out of 18 controls (owing to intubation errors), 0 out of 18 at the lowest dose, 0 out of 18 at the intermediate dose and 1 out of 18 at the highest dose. A dose-related increase (not statistically evaluated) in the rate of early (mainly) and late resorptions was seen at the two higher doses (mean of 0.4, 0.6, 1.6 and 1.6 resorptions/litter, historical control litter average was 0.6, range: 0.2–1.0 resorptions). Fetuses at these doses (two fetuses from two litters at the highest dose and one fetus at the intermediate dose) also had dose-related increased incidences of domed head, hydrocephalus and marked enlargement of the fontanelle: fetal incidence, 0 out of 95, 0 out of 124, 1 out of 92 and 2 out of 56 (historical control incidence, 0.1%; range, 0–1.1%); litter incidence, 0 out of 14, 0 out of 16, 1 out of 14 and 2 out of 9 (historical control incidence, 0.7%; range, 0–8.3%). The NOAEL for maternal and developmental toxicity was 24 mg/kg bw per day (Hoberman, 1989; Lankas & Wise, 1993).

In another study of developmental toxicity, evaluated by JECFA in 1992 (JECFA, 1993) and re-evaluated by the present Meeting, groups of 18 pregnant New Zealand White rabbits received thiabendazole (purity, 98.6%) at a dose of 0, 50, 150 or 600 mg/kg bw per day in 0.5% methylcellulose by gavage on days 6–18 of gestation. The animals were killed on day 28 of gestation. Effects were seen only at the highest dose; they comprised decreased maternal food consumption (up to 20–30% less than control values on days 13–19) and weight gain (69% less than control value), increased rates of early and late resorptions (statistically significant increase in total resorptions, 11.8% compared with 8.1% in controls), decreased fetal body weight (11% and 13% less than control values in females and males, respectively) and increased incidences of common variation in lung lobation (11% compared with 1% in controls) and incompletely ossified metacarpals (24% compared with 5% in controls). No evidence for compound-related fetal hydrocephaly was found. The NOAEL for maternal and developmental toxicity was 150 mg/kg bw per day (Wise & Lankas, 1991 and Lankas & Wise, 1993).

3. Observations in humans

In a study in volunteers, 50 men aged 20–57 years received capsules containing placebo and 50 received 125 mg of thiabendazole twice per day for 24 weeks. Neither the study subjects nor the investigators were aware of who had received placebo. In total, 36 men receiving thiabendazole and 41 receiving placebo completed the study. One man was removed from the study at his own request because of daytime sedation and markedly decreased energy. The other withdrawals were clearly unrelated to the treatment. Weekly interviews were conducted to record side-effects. General physical examinations and laboratory examinations (haematology, measurement of cholesterol, glucose, urea, alkaline phosphatase, thymol turbidity, bilirubin in serum and urine analysis) were carried out before the test and after 4, 12 and 24 weeks. The haematological parameters measured were complete blood count including erythrocyte volume fraction. Protein-bound iodine in serum and electrocardiographic traces were evaluated only at the beginning and after 24 weeks of the study.

This study was previously summarized by the 1977 JMPR which noted that, under the conditions of the study, thiabendazole was well tolerated, and no effect on any of the parameters measured could be clearly ascribed to treatment. The JMPR identified a no-observed-effect level (NOEL) of 3–4 mg/kg bw per day, which was confirmed by JECFA in 1992 (JECFA, 1993). The following observations may be relevant for the acute toxicity of thiabendazole, although the time of onset of clinical signs was not specified. The men reported the following possible side-effects (treated

versus placebo): increased appetite (26 out of 50 vs 30 out of 50), flatulence (6 out of 50 vs 3 out of 50), nausea (4 out of 50 vs 2 out of 50), increased urinary frequency (3 out of 50 vs 3 out of 50) and sedation (7 out of 50 vs 5 out of 50) (Colmore, 1965).

In a review of studies, previously summarized by JECFA in 2002 (JECFA, 2002), on the efficacy of thiabendazole against parasites in humans, the standard therapeutic oral dose was 25 mg/kg bw twice per day for 1–4 days, although higher doses were used in some studies. The incidences of minor transient side-effects were generally 25–30% with the standard dose and higher with higher doses. The effects comprised anorexia, nausea, vomiting and dizziness. Serious side-effects were rare and comprised numbness, collapse, tinnitus, abnormal sensation in the eyes, xanthopsia, enuresis, decreased pulse rate and systolic blood pressure and transient rises in the frequency of cephalin flocculation (emulsions of cephalin are readily precipitated when mixed with serum of patients suffering from hepatitis and allied disorders of the liver, therefore this test is used for the recognition of certain hepatic diseases) and in aspartate aminotransferase activity (Campbell & Cuckler, 1969).

Side-effects in humans after therapeutic oral doses (not specified) of thiabendazole were reported in another literature review, previously summarized by JECFA in 2002 (JECFA, 2002). Common effects were dizziness (the frequency ranging from < 5% to 80%, depending on dosage) and nausea and vomiting (5–15%). Rarely observed side-effects included anorexia, abdominal pain, headache, drowsiness, weariness, heartburn, diarrhoea or constipation, flatulence, blurring of vision, xanthopsia, skin eruption, malodorous urine and vomiting of live *Ascaris*. The extent to which these frequencies differed from those in untreated subjects is unknown, although in two placebo-controlled studies, dizziness was reported to be approximately twice as common in thiabendazole-treated subjects as in placebo-treated subjects (Cuckler & Mezey, 1966).

In a clinical case report, previously summarized by JECFA in 2002 (JECFA, 2002), the following side-effects were reported in 14 of 23 patients with trichinosis who had received thiabendazole orally at a dose of 50 mg/kg bw as two daily doses for 10 days: nausea (11 out of 23), retching (11 out of 23), vomiting (11 out of 23), aversion to tablets (3 out of 23), exanthema (3 out of 23), impotence (2 out of 23), diarrhoea (1 out of 23), liver damage (1 out of 23), fever (1 out of 23) and dizziness (1 out of 23) (Hennekeuser et al., 1969). Again, the incidence of side-effects in untreated patients was not reported, and the extent to which these effects might have been influenced by the underlying condition was not reported.

From these surveillance data it is difficult to discern which of the effects are due solely to thiabendazole, or to patients' allergic or inflammatory responses resulting from the killing of microflorae, referred to as the Mazotti reaction, or to other possible non-drug effectors.

Comments

Previously evaluated studies

Studies to establish median lethal doses of thiabendazole given orally (LD_{50} values > 2000 mg/kg bw) did not provide any indication of acute effects.

In 2002, JECFA considered that emesis in dogs and effects on the kidney, haematopoietic system and development were relevant end-points for establishing an ARfD.

Clinical effects: In dogs, the NOAEL for emesis was 40 mg/kg bw per day. Common side-effects reported in humans receiving therapeutic doses (25 mg/kg bw or greater, twice per day for 1–10 days)

included anorexia, nausea, vomiting and dizziness. In a study in volunteers, in which controls were given a placebo, a dose of 125 mg of thiabendazole given twice per day for 24 weeks (equivalent to 3.6 mg/kg bw per day for a 60 kg person) did not cause significant changes in subjective side-effects.

Kidney effects: In single-dose studies in mice, renal toxicity, mainly in the proximal tubules, was observed at doses of 250 mg/kg bw and higher, and consisted of histopathological changes including mitochondrial swelling and ultimately necrosis of epithelial cells. Effects were most severe 2–3 days after dosing; after that time, tissue repair processes began. Apart from tubular dilatation, all effects were either fully or partly reversed within 10 days of administration. The NOAEL was 125 mg/kg bw.

Haematological effects: Changes indicative of anaemia were occasionally seen early in 4- or 13-week studies in rats and 14- and 53-week studies in dogs. As histopathological changes indicative of anaemia occurred after one or several doses, they were considered by the 2002 JECFA to be relevant for assessing acute exposure. The NOAELs for these effects in rats and dogs were 9 and 10 mg/kg bw per day, respectively. However, in single-dose studies in rats treated by gavage assessed by the present Meeting, no treatment-related changes in haematology parameters were observed at up to 1000 mg/kg bw, the highest dose tested.

In a study in volunteers, 50 men received an oral dose of 125 mg of thiabendazole twice per day for 24 weeks (equivalent to 3.6 mg/kg bw per day for a 60 kg person), and 50 other men were given a placebo. Thiabendazole did not affect haematological parameters in blood after 4, 12 or 24 weeks of treatment. However, histopathological examinations, which in animals were more sensitive indicators of haematotoxicity, were obviously not performed.

Developmental effects: As teratogenic effects and early resorptions may be induced by a single dose within a certain sensitive period, these effects on the fetus are particularly relevant to setting an ARfD. Five studies were provided for assessment by the present Meeting. In a published study in mice, teratogenic effects were observed after a single oral dose on day 9 of gestation. These effects consisted of deformed limbs at doses of 480 mg/kg bw and higher (NOAEL, 270 mg/kg bw) and fusion of vertebrae and ribs at 240 mg/kg bw and higher (NOAEL, 130 mg/kg bw). Excessive maternal mortality and lack of data on other maternally toxic effects compromised the interpretation of this study and the present Meeting considered this study as supplementary information only. In another study in mice, no teratogenic effects were observed when thiabendazole was given at doses of up to 200 mg/kg bw per day on days 6–15 of gestation. Thiabendazole was not teratogenic in rats at doses of up to 80 mg/kg bw per day, the highest dose tested.

Increased rates of resorption were observed in mice and rabbits, but not in rats. In mice, the NOAEL for this effect was 700 mg/kg bw per day when the animals were treated by gavage on days 7–15 of gestation, and 1400 mg/kg bw after a single dose administered by gavage on day 9 of gestation. Rabbits exposed on days 6–18 of gestation showed increased rates of resorption (mainly early resorption) at oral doses of 120 mg/kg bw per day and higher, with a NOAEL of 24 mg/kg bw per day. In another study in rabbits, rates of resorption were increased at 600 mg/kg bw per day, with a NOAEL of 150 mg/kg bw per day.

In another study in mice treated by gavage on days 6–15 of gestation, decreases in the number of implantations and in the number of live fetuses were observed at doses of 100 mg/kg bw per day and higher. The NOAEL was 25 mg/kg bw per day. The effects on implantation were considered to result from a direct effect of the substance since they were seen within the first few days after treatment, before maternal toxicity (decrease in food consumption and body weight) was observed.

Studies evaluated for the first time at this meeting

Single-dose studies of toxicity: Three single-dose studies of toxicity in rats were provided for assessment by the present Meeting. In the gavage studies, dose-related effects including reduced

activity, tiptoe gait, landing foot splay and reduced motor activity were observed for up to 24 h after 100 or 200 mg/kg bw and for up to 3 days after 1000 mg/kg bw. At this, the highest dose, there was also a transient reduction in body weight compared with controls. There were no treatment-related changes in haematology parameters. As the neuroactive effects observed at 100 mg/kg bw were marginal, the NOAEL was 100 mg/kg bw. In the dietary study, no treatment-related effects on clinical signs, FOB assessment, motor activity or body weight were observed at up to 600 ppm (equal to 46 mg/kg bw), the highest dose tested.

Toxicokinetic studies: Toxicokinetic studies that compared the gavage and dietary routes of administration showed that different kinetic profiles of thiabendazole were obtained, particularly with respect to C_{max}, which was shown to be much higher by the gavage route than by the dietary route. The administration of an aqueous slurry of diet as a model for residues of thiabendazole in food commodities containing a high residue of thiabendazole demonstrated that by this more relevant route of exposure the kinetic behaviour of thiabendazole was closer to the situation seen in the dietary study. Therefore the results of the dietary study would be more appropriate for deriving the ARfD. However, the substance was not tested at doses high enough to produce any toxic effects.

Toxicological evaluation

After considering the data available to the present Meeting as well as the 2002 JECFA evaluation, the Meeting established an ARfD of 0.3 mg/kg bw for women of childbearing age on the basis of the NOAEL of 25 mg/kg bw per day identified on the basis of reduction of implantations at doses of 100 mg/kg bw per day and higher in a study of developmental toxicity in mice, and a safety factor of 100. This value was supported by a NOAEL of 24 mg/kg bw per day identified on the basis of increases in resorptions at doses of 120 mg/kg bw per day and greater in a study of developmental toxicity in rabbits.

The Meeting established an ARfD of 1 mg/kg bw for the general population on the basis of a NOAEL of 100 mg/kg bw identified on the basis of some slight neuroactive effects at doses of 200 mg/kg bw and greater in a study of acute toxicity in rats treated by gavage, and a safety factor of 100.

Levels relevant to acute dietary risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Developmental toxicity[b]	Maternal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
		Developmental toxicity	25 mg/kg bw per day	100 mg/kg bw per day
Rat	Single-dose toxicity study	Toxicity	100 mg/kg bwb	200 mg/kg bw
			600 ppm, equal to 46 mg/kg bw[a,c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	24 mg/kg bw per day	120 mg/kg bw per day
		Developmental toxicity	24 mg/kg bw per day	120 mg/kg bw per day

[a] Dietary administration
[b] Gavage administration
[c] Highest dose tested

Estimate of acute reference dose

0.3 mg/kg bw for women of childbearing age

1 mg/kg bw for the general population

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

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

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