THIABENDAZOLE (addendum)

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Explanation

Thiabendazole (2-(4-thiazolyl)-1*H*-benzimidazole) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2006, when an acceptable daily intake (ADI) of 0–0.1 mg/kg body weight (bw) was established. The 2006 Meeting also established an acute reference dose (ARfD) of 1 mg/kg bw for the general population and an ARfD of 0.3 mg/kg bw for women of childbearing age (Annex 1, reference *109*).

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, thiabendazole was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review.

Several toxicological studies on thiabendazole were submitted to the present Meeting, including an acute neurotoxicity study, a 90-day neurotoxicity study and an immunotoxicity study.

All critical studies contained statements of compliance with good laboratory practice and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. One additional study that complemented the toxicological information submitted for the current assessment was identified from a literature search and was included in the evaluation.

Evaluation for acceptable intake

1. Toxicological studies

1.1 Long-term studies of toxicity and carcinogenicity

A carcinogenicity study, obtained from the open literature, was conducted using ICR mice (50 of each sex per group) treated with thiabendazole in the diet at a concentration of 0, 310, 1250 or 5000 parts per million (ppm) (equal to 0, 33.2, 146 and 605 mg/kg bw per day for males and 0, 40.0, 179 and 615 mg/kg bw per day for females, respectively) for 78 weeks.

There was no treatment-related effect on mortality in either sex. Body weights were depressed at 1250 ppm and above. The depression at 5000 ppm was more severe, approximately 20% and 15% for males and females, respectively. No treatment-related effects on haematology were observed. Major

treatment-related changes were found in the kidney at 1250 ppm and above and in the urinary bladder at 5000 ppm. At 1250 ppm, the incidence of nephrosis was increased in both sexes. At 5000 ppm, the incidences of calculi in the kidney and diffuse hyperplasia of urinary epithelium in the renal papilla and urinary bladder were increased. No treatment-related increase in tumours in any tissue was observed.

The no-observed-adverse-effect level (NOAEL) for long-term toxicity in mice was 310 ppm (equal to 33.2 mg/kg bw per day), based on body weight suppression and an increased incidence of nephrosis at 1250 ppm (equal to 146 mg/kg bw per day). No carcinogenicity was observed (Tada et al., 2001).

1.2 Special studies

(a) Acute neurotoxicity

An acute neurotoxicity study was conducted in non-fasted Crl:CD(SD) rats (10 of each sex per group) treated with thiabendazole (purity 99.3%; lot/batch no. H1998/011) in an aqueous 0.5% carboxymethylcellulose vehicle as a single gavage dose of 0, 50, 200 or 2000 mg/kg bw. The dosing volume was 5 mL/kg bw for all groups. Functional observational battery (FOB) assessments and locomotor activity (LMA) data were recorded for all animals prior to the initiation of dose administration, at time of peak effect (approximately 3 hours following dose administration) on study day 0, and on study days 7 and 14. On study day 15, rats were euthanized and necropsied.

All animals survived. There were no test substance–related clinical findings during the daily observations. Treatment-related changes in body weight were observed at 200 mg/kg bw and above in both sexes (Table 1). Body weights and body weight gains within 4 days after the treatment were statistically significantly decreased in both sexes at 2000 mg/kg bw, and body weight gains 1 day after the treatment were statistically significantly decreased in both sexes at 200 mg/kg bw. At 50 mg/kg bw, body weight gain 1 day after the treatment was slightly (approximately 2.5%), but also statistically significantly, decreased in females only. Statistically significant decreases in body weight or body weight gain within 4 days after the treatment were not observed in either sex at 50 mg/kg bw. This transient and slight decrease in body weight gain, which was not associated with other adverse effects in females at 50 mg/kg bw, was not considered to be an adverse effect of treatment. At 2000 and 200 mg/kg bw, feed consumption was decreased on the day of dosing (Table 1); subsequently, feed consumption was reduced at 2000 mg/kg bw only.

Treatment-related FOB findings were limited to the 200 and 2000 mg/kg bw males and females at time of peak effect (approximately 3 hours post-dosing) and included slight lacrimation and slightly drooping eyelids (2000 mg/kg bw females), decreased mean rearing counts (2000 mg/kg bw males and 200 and 2000 mg/kg bw females) and increased mean time to first step (2000 mg/kg bw females). The FOB findings were transient, as no test substance–related findings for these parameters were observed on study days 7 and 14. There were no FOB findings at 50 mg/kg bw.

No treatment-related findings were observed in sensory, neuromuscular or physiological observations or histopathological examinations.

LMA counts were varied, but statistically significant changes were observed on day 1 only (Table 1). The cumulative total LMA counts decreased in both sexes at 200 mg/kg bw and above, and the cumulative ambulatory LMA counts also decreased in males at 50 mg/kg bw and above and in females at 200 mg/kg bw and above. The decreases in ambulatory LMA counts were limited to within the first 20 minutes at 200 mg/kg bw and above, and no statistically significant decrease in ambulatory LMA count was found after 20 minutes except in the 2000 mg/kg bw group. The cumulative total LMA counts were comparable after day 1. The decreases observed at 200 mg/kg bw in both sexes within the first 20 minutes were slight but considered treatment related as a result of other treatment-related changes found at these doses.

A decrease in ambulatory LMA counts was noted in females at 50 mg/kg bw within 10 minutes, but no difference was found in subsequent subintervals on day 1 or the cumulative counts on day 1. The

decrease in ambulatory LMA counts in males at 50 mg/kg bw was not observed during the first 10 minutes and was not consistent with the decreases found at 200 mg/kg bw and above.

Table 1. Summary of acute neurotoxicity study in rats treated with thiabendazole

	Males				Females			
Parameter	**0 mg/kg bw**	**50 mg/kg bw**	**200 mg/kg bw**	**2 000 mg/kg bw**	**0 mg/kg bw**	**50 mg/kg bw**	**200 mg/kg bw**	**2 000 mg/kg bw**
Body weight gain (g) during days 0–1[a]	5 ± 2.3	1 ± 2.9	−7 ± 5.3**	−13 ± 6.8**	3 ± 3.3	−1 ± 2.5*	−6 ± 3.3**	−8 ± 3.6**
Body weight gain (g) during days 1–2[a]	8 ± 3.3	9 ± 3.5	12 ± 3.5**	−5 ± 3.6**	5 ± 2.6	4 ± 5.3	6 ± 2.3	−6 ± 3.5*
Body weight gain (g) during days 7–8[a]	7 ± 1.4	6 ± 3.7	6 ± 4.5	7 ± 2.3	2 ± 5.6	2 ± 4.1	2 ± 5.2	2 ± 3.6
Feed consumption on day of dosing (g/rat per day)	21	19	13**	8**	16	14	9**	6**
Total locomotor activity counts[a]								
Pretest (cumulative[b])	2 010 ± 478	2 542 ± 581	2 341 ± 396	1 956 ± 536	2 237 ± 434	2 180 ± 443	1 846 ± 637	2 043 ± 600
Day 1[c] (cumulative[b])	2 178 ± 761	1 705 ± 667	1 333 ± 387**	1 069 ± 455**	2 524 ± 622	2 362 ± 776	1 586 ± 530**	926 ± 477**
Day 7 (cumulative[b])	2 428 ± 107	2 616 ± 533	2 519 ± 467	1 852 ± 767	2 710 ± 1 125	3 223 ± 1 121	2 963 ± 950	3 016 ± 758
Ambulatory locomotor activity counts[a]								
Pretest (cumulative[b])	475 ± 181	560 ± 212	593 ± 234	479 ± 198	584 ± 199	575 ± 157	466 ± 188	519 ± 239
Day 1[c] (cumulative[b])	550 ± 232	388 ± 150*	299 ± 125**	241 ± 124**	730 ± 185	609 ± 205	394 ± 143**	238 ± 137**
Subinterval on day 1[c]								
0–10 minutes	375 ± 111	305 ± 105	222 ± 87**	136 ± 101**	565 ± 121	417 ± 103**	305 ± 106**	121 ± 82**
11–20 minutes	128 ± 114	62 ± 53*	24 ± 31**	22 ± 26**	102 ± 106	105 ± 79	35 ± 57**	17 ± 28*
21–30 minutes	25 ± 37	8 ± 18	19 ± 29	24 ± 22	42 ± 62	72 ± 90	32 ± 68	12 ± 18
31–40 minutes	7 ± 14	5 ± 9	16 ± 23	25 ± 24	12 ± 32	9 ± 21	10 ± 19	45 ± 71
41–50 minutes	0 ± 1	8 ± 22	14 ± 23	14 ± 15	1 ± 4	5 ± 14	9 ± 28	18 ± 19*
51–60 minutes	14 ± 42	1 ± 2	5 ± 9	20 ± 15	8 ± 16	0 ± 0.4	3 ± 8	24 ± 23*
Day 7 (cumulative[b])	511 ± 238	593 ± 186	568 ± 224	353 ± 176	793 ± 503	814 ± 308	811 ± 322	759 ± 210

bw: body weight; *: $P \leq 0.05$; **: $P \leq 0.01$

[a] All changes are expressed as mean ± standard deviation.

[b] Cumulative during 0–60 minutes.

[c] Three hours after dosing.

Source: Herberth (2012a)

The decreases in the ambulatory LMA counts at 50 mg/kg bw on study day 0 were not considered adverse for the following reasons:

1) The results seen at 50 mg/kg bw were within the range of the concurrent control values, which showed the high level of variability typically seen in these measurements.
2) The decrease observed at 50 mg/kg bw was transient at subintervals of 11–20 minutes in males and 0–10 minutes in females on day 0. This transient decrease did not result in a statistically significant decrease in the cumulative LMA counts in females at 50 mg/kg bw on day 1.
3) No treatment-related neurological effects were observed at 50 mg/kg bw.

The NOAEL for systemic toxicity was 50 mg/kg bw, based on decreases in mean rearing counts in females, body weight loss secondary to reduced feed consumption, and lower ambulatory LMA counts at the time of peak effect on study day 0 in both sexes at 200 mg/kg bw. There was no clear evidence that thiabendazole was acutely neurotoxic (Herberth, 2012a).

(b) Short-term studies of neurotoxicity

In a 90-day neurotoxicity study, thiabendazole (purity 99.3%; lot/batch no. HK1998/011) was administered to Crl:CD(SD) rats (12 of each sex per group) in the diet at 0, 200, 750 or 1500 ppm (equal to 0, 13, 47 and 95 mg/kg bw per day for males and 0, 15, 54 and 108 mg/kg bw per day for females, respectively). FOB and LMA data were recorded for all animals during the pretest and then during the second, fourth, eighth and thirteenth weeks of test diet administration (study weeks 1, 3, 7 and 12, respectively). Ophthalmic examinations were performed. Five rats of each sex per group were deeply anaesthetized and perfused in situ during study week 13; brain weights and brain dimensions (excluding olfactory bulbs) were recorded. Neuropathological examination of the rats in the 0 and 1500 ppm groups was performed after perfusion fixation.

All animals survived to the scheduled euthanasia. There were no test substance–related clinical findings during the weekly examinations.

Treatment-related lower mean body weight gains, with corresponding decrements in feed consumption, were generally noted for the 1500 ppm males and females throughout the exposure period. These changes were occasionally statistically significant, resulting in a statistically significantly lower overall (study days 0–91) mean body weight gain for both males (11.3% on day 91) and females (17.4% on day 91) at 1500 ppm. As a result, mean absolute body weights for males and females were lower than the control values beginning on study day 7 and continuing throughout the study; the differences were statistically significant during study days 84–91 for males (18.3%) and study days 14–91 for females (36.1%). Feed consumption was lower in males (8%) and females (17%) at 1500 ppm throughout the study.

No treatment-related changes were observed in clinical signs, FOB evaluations, mean total and ambulatory LMA counts, the pattern of habituation, ophthalmology, neuropathology, and weights and measurements of the brain in either sex in any treatment group.

The NOAEL for systemic toxicity was 750 ppm (equal to 47 mg/kg bw per day), based on decreased body weight gains, lower body weights and decreased feed consumption at 1500 ppm (equal to 95 mg/kg bw per day).

The NOAEL for neurotoxicity was 1500 ppm (equal to 95 mg/kg bw per day), the highest dose tested (Herberth, 2012b).

(c) Immunotoxicity

In an immunotoxicity study, thiabendazole (purity 99.3%; batch no. HK1998/011) was administered to female CD-1 mice (10 per group) in the diet at a concentration of 0, 100, 1000 or 5000

ppm (equal to 0, 20.9, 205.6 and 1027.0 mg/kg bw per day, respectively) for 28 days. The concurrent vehicle control group and the positive control group were offered the basal (untreated) diet on a comparable regimen to the thiabendazole-treated groups. All mice received an intravenous injection of sheep red blood cells on study day 24. Mice in the positive control group were administered the positive control substance, cyclophosphamide, via intraperitoneal injection (50 mg/kg bw per day) once daily for 4 days (study days 24 through 27). All animals were euthanized on study day 28. Histopathological examination was not conducted.

Lower body weights were observed in the 5000 ppm group from study days 0 to 3, but the values were generally similar to those of the vehicle control group for the remainder of the study. Thiabendazole-related increases in absolute liver weights were noted in the 1000 and 5000 ppm groups; however, when adjusted for terminal body weight, statistically significantly higher (41.2%) liver weight was noted only at 5000 ppm.

In the functional immune evaluation of the immunoglobulin M (IgM) antibody-forming cell (AFC) response, there was a thiabendazole-related lower total spleen activity, measured as IgM antibody-forming cells per spleen (AFC/spleen), at 5000 ppm (53%) when compared with the vehicle control group; however, thiabendazole did not significantly suppress spleen cell numbers or specific IgM antibody-forming cell activity (AFC/10^6 spleen cells) at any dose.

For the positive control (cyclophosphamide) group, statistically significantly lower spleen and thymus weights, spleen cell numbers, specific activity and total spleen activity of IgM antibody-forming cells were noted when compared with the vehicle control group; these effects were consistent with the known immunosuppressant effects of cyclophosphamide and validated the functionality of the assay.

The NOAEL for immunotoxicity was 1000 ppm (equal to 205.6 mg/kg bw per day), based on lower total spleen activity measured as IgM antibody-forming cells per spleen at 5000 ppm (equal to 1027.0 mg/kg bw per day).

The NOAEL for systemic toxicity was 1000 ppm (equal to 205.6 mg/kg bw per day), based on lower body weights and markedly increased liver weights at 5000 ppm (equal to 1027.0 mg/kg bw per day) (Wasil, 2012).

Comments

Toxicological data

In a chronic toxicity and carcinogenicity study, mice were administered thiabendazole in the diet at 0, 310, 1250 or 5000 ppm (equal to 0, 33.2, 146 and 605 mg/kg bw per day for males and 0, 40.0, 179 and 615 mg/kg bw per day for females, respectively) for 78 weeks. The NOAEL for long-term toxicity in mice was 310 ppm (equal to 33.2 mg/kg bw per day), based on body weight suppression and an increased incidence of nephrosis at 1250 ppm (equal to 146 mg/kg bw per day). No carcinogenicity was observed (Tada et al., 2001).

In an acute neurotoxicity study in rats treated with thiabendazole as a single dose of 0, 50, 200 or 2000 mg/kg bw by gavage, the NOAEL for systemic toxicity was 50 mg/kg bw, based on decreases in mean rearing counts in females, body weight loss secondary to reduced feed consumption, and lower ambulatory LMA counts at the time of peak effect on study day 0 in both sexes at 200 mg/kg bw. There was no clear evidence that thiabendazole was acutely neurotoxic (Herberth, 2012a).

In a 90-day neurotoxicity study in rats treated with thiabendazole in the diet at 0, 200, 750 or 1500 ppm (equal to 0, 13, 47 and 95 mg/kg bw per day for males and 0, 15, 54 and 108 mg/kg bw per day for females, respectively), the NOAEL for systemic toxicity was 750 ppm (equal to 47 mg/kg bw per day), based on findings of decreased body weight gain, depressed body weights and lower feed consumption at 1500 ppm (equal to 95 mg/kg bw per day). The NOAEL for neurotoxicity was 1500 ppm (equal to 95 mg/kg bw per day), the highest dose tested (Herberth, 2012b).

The Meeting concluded that thiabendazole is not neurotoxic.

In an immunotoxicity study in female mice treated with thiabendazole in the diet at a concentration of 0, 100, 1000 or 5000 ppm (equal to 0, 20.9, 205.6 and 1027.0 mg/kg bw per day, respectively) for 28 days, the NOAEL for immunotoxicity was 1000 ppm (equal to 205.6 mg/kg bw per day), on the basis of lower total spleen activity measured as IgM antibody-forming cells per spleen at 5000 ppm (equal to 1027.0 mg/kg bw per day). The NOAEL for systemic toxicity was 1000 ppm (equal to 205.6 mg/kg bw per day), based on reduced body weight and marked increases in liver weights at 5000 ppm (equal to 1027.0 mg/kg bw per day) (Wasil, 2012).

The Meeting concluded that thiabendazole is not immunotoxic in the absence of systemic toxicity.

Toxicological evaluation

The Meeting concluded that no revision of the ADI or ARfDs was necessary. The Meeting noted that the NOAEL for systemic toxicity from the acute neurotoxicity study (50 mg/kg bw) is lower than the NOAEL from the study currently used in the derivation of the ARfD for the general population (100 mg/kg bw). However, as the lowest-observed-adverse-effect level (LOAEL) for both studies is 200 mg/kg bw, and as the findings in both studies are similar, the Meeting concluded that there was no reason to revise the ARfD for the general population.

Critical end-points for setting guidance values for exposure to thiabendazole

Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight suppression, nephrosis
Lowest relevant NOAEL	33.2 mg/kg bw per day (mouse)
Carcinogenicity	No evidence of carcinogenicity
Neurotoxicity	
Acute neurotoxicity NOAEL	2000 mg/kg bw, highest dose tested (rat) Systemic toxicity NOAEL 50 mg/kg bw (rat)
Ninety-day neurotoxicity NOAEL	95 mg/kg bw per day, highest dose tested (rat)
Other toxicological studies	
Immunotoxicity NOAEL	205.6 mg/kg bw per day (mouse); not immunotoxic in the absence of systemic toxicity

References

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Tada Y, Fujitani T, Yano N, Yuzawa K, Nagasawa A, Aoki N et al. (2001). Chronic toxicity of thiabendazole (TBZ) in CD-1 mice. Toxicology. 169(3):163–76.

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