

CARBENDAZIM (addendum)

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Explanation

Carbendazim is the International Standardization Organization (ISO) approved common name for methyl 2-benzimidazole carbamate, a systemically active benzimidazole fungicide that inhibits the synthesis of β -tubulin. Carbendazim was previously evaluated by the Joint Meeting in 1973, 1977, 1983, 1985, and 1995. In 1995, an acceptable daily intake (ADI) of 0–0.03 mg/kg bw was established based on the no-observed-adverse-effect level (NOAEL) of 2.5 mg/kg bw per day in a 2-year study in dogs and a safety factor of 100.

The Meeting had been asked by the Codex Committee on Pesticide Residues to consider the need for an acute reference dose (ARfD) for carbendazim. The present Meeting therefore evaluated relevant original studies that had been considered by previous Meetings, and newly submitted data on genotoxicity and reproductive toxicity.

Evaluation for an acute reference dose

1. Toxicological studies

1.1 Short-term studies of toxicity

Rats

In a dose range-finding study, groups of 10 male and 10 female Wistar rats received diets containing carbendazim (purity not indicated) at a concentration of 0, 80, 400, 2000, 10 000 or 50 000 ppm (equivalent to 0, 8, 40, 200, 1000, and 5000 mg/kg bw per day) for 30 days. A

decrease in body weight and weight gain was observed at the two higher doses. At the highest dose, feed consumption was reduced, and eight males and eight females died with severe emaciation. Surviving animals at this dose showed leukopenia, siderosis in liver and kidneys and arrest of spermatogenesis. Inhibition of spermatogenesis was also observed in three animals at 10 000 ppm. The NOAEL was 2000 ppm, equivalent to 200 mg/kg bw per day, on the basis of reduced body-weight gain and inhibition of spermatogenesis at 10 000 ppm and above (Scholz & Weigand, 1972).

In a short-term study of toxicity, groups of 20 male and 20 female Wistar rats received diets containing carbendazim (purity not indicated) at a concentration of 0, 80, 400, 2000 or 10 000 ppm (equal to 6.5, 32, 163, and 780 mg/kg bw per day in males and 6.9, 36, 174, and 847 mg/kg bw per day in females) for 93 days. Half of the animals in each group were sacrificed, and the remaining rats were fed untreated diet for a 12- to 14-day recovery period. Body weight was reduced at the highest dose, but no effects were observed on feed consumption. No clinical signs of toxicity were observed. After 93 days of feeding, relative liver weights were increased in females at ≥ 400 ppm and in males at ≥ 2000 ppm. These changes were not evident after the recovery period. No histopathological lesions or liver enzyme changes were noted. One male at 10 000 ppm had small testes and atrophy of the seminiferous tubuli. The NOAEL was 2000 ppm, equal to 163 mg/kg bw per day, on the basis of reduced body weight at 10 000 ppm (Scholz & Schultes, 1973).

Dogs

In a range-finding study, groups of two male and two female juvenile beagle dogs were fed carbendazim (purity not indicated) at a dietary concentration of 0, 500, or 2500 ppm (equal to 0, 19–21, and 96–99 mg/kg bw per day, depending on sex) for 28 days. Body weight, development, feed consumption, and overall health were not affected by treatment. Liver weights were increased in females at 500 and 2500 ppm (161% and 216% of controls, respectively), and activity of serum glutamic-pyruvic transaminase and alkaline phosphatase were increased in males at the highest dose. Pathological changes were confined to the liver of males and females at 2500 ppm. In males, the damage was mainly characterized by peliosis-like changes, bile-duct proliferation, accumulations of reticulo-endothelial cells, and periportal cellular infiltration. In females, the changes consisted of greatly enlarged hepatocytes with a watery pale-staining cytoplasm. The NOAEL was 500 ppm, equal to 19–21 mg/kg bw per day, on the basis of liver toxicity at 2500 ppm (Til et al., 1971).

1.2 Studies of developmental toxicity

In a study of prenatal developmental toxicity, groups of 27–28 pregnant ChR-CD rats were fed diets containing carbendazim as a formulation (carbendazim, 53%) at a concentration of 0, 100, 500, 2500, 5000, 7500, or 10 000 ppm (equal to 0, 8.9, 46, 218, 432, 626 and 747 mg/kg bw per day from day 6 to day 15 of gestation). The animals were received in three shipments at three different times; however, data were presented as a unit. Animals in the second control group and in the groups receiving the two higher doses were received in a single shipment. After day 15, rats received control diet until sacrifice on day 20, when litters were delivered by caesarean section. The numbers and location of live and dead fetuses and resorption sites, body weights, crown-rump length, sex, and visible abnormalities were determined. Two thirds of the fetuses were examined for skeletal defects, and the remainder were examined for visceral and soft-tissue anomalies.

There were no deaths or treatment-related signs of toxicity during the study. Body weight and body-weight gain were not adversely affected by treatment. A slight decrease in feed consumption at 10 000 ppm was noted during the dosing period (Table 1). Reproductive parameters (number of implantation and resorption sites, number of females with complete or

partial resorption, number of live fetuses and dead fetuses) were not affected by treatment. Fetal body weights, crown–rump length and the incidences of gross external, skeletal, and soft tissue malformations and variations were similar in all groups. Three fetuses were found with malformations: one at 2500 ppm (meningoencephalocele, absence and retarded ossification of cranial bones), one at 5000 ppm (meningoencephalocele with unilateral microphthalmia and misshapen premaxillary and maxillary bones), and one at 10 000 ppm (bilateral agenesis, absence and fusion of several ribs and corresponding thoracic vertebrae, absence of the atlas).

There was no evidence for embryo- or fetotoxicity or teratogenicity when pregnant rats were given feed containing carbendazim at concentrations of up to 10 000 ppm, equal to 747 mg/kg bw per day. The NOAEL for maternal and developmental toxicity was 10 000 ppm (equal to 747 mg/kg bw per day), the highest dose tested (Culik et al., 1970).

In a study of prenatal developmental toxicity, groups of 15–26 pregnant Sprague-Dawley rats were given carbendazim (purity not indicated; technical active substance) at a dose of 10, 30, 60, 100, 300, 1000 or 3000 mg/kg bw per day by oral gavage in a 0.5% aqueous suspension of carboxymethylcellulose on days 6–15 of gestation, while two control groups of 27 or 51 animals were untreated or received the vehicle only. The study complied with Food and Drug Agency (FDA) guidelines for 1966, and the investigations were carried out between April and July 1974. The dams were killed on day 20 of gestation, and the reproductive tract was examined for corpora

Table 1. Selected findings from a study of prenatal developmental toxicity in rats

Finding	Dietary concentration (ppm)							
	0 (control group 1)	0 (control group 2) ^a	100	500	2500	5000	7500 ^a	10 000 ^a
No. of pregnant animals	28	23	24	22	26	27	22	25
Body weight (g):								
Day 6	210	208	208	204	207	201	221	219
Day 16	277	289	282	279	283	267	285	271
Day 20	354	351	353	348	356	337	352	348
Feed intake (g/rat per day):								
Days 6–16	21.9	23.6	21.8	22.2	21.4	20.2	21.1	18.3
Days 16–20	26.0	27.4	25.1	27.0	26.6	26.3	30.0	29.1
No. of implantations/dam	9.6	10.0	10.8	9.8	10.4	9.9	10.2	10.9
Postimplantation loss (%)	4.5	7.8	8.9	6.0	4.7	5.6	5.2	4.8
No. of live fetuses/dam	9.2	9.2	10.0	9.2	10.1	9.4	9.4	10.4
Mean fetal weight (g)	3.89	3.76	3.99	3.88	4.15	3.95	3.68	3.63
No. of live fetuses	257	212	239	202	263	253	209	259
No. of malformed fetuses	0	0	0	0	1	1	0	1
Sternebrae unossified (% fetuses)	9.5	18.1	6.2	19.5	11.6	8.3	16.4	13.9
14th rib(s) (% fetuses)	8.9	9.4	23.6	15.8	15.1	15.9	23.5	16.2
Hydronephrosis (% fetuses)	15.9	24.3	NE	NE	NE	16.7	15.6	9.3

From Culik et al. (1970)

^a Animals received in one shipment

NE, not examined.

lutea, implantation and resorption sites, and live and dead fetuses. The fetuses were weighed, sexed and examined for external abnormalities. Two thirds of the live fetuses from each litter were selected at random and examined for skeletal alterations using a modification of the Dawson method, the remaining part of each litter was prepared and examined for soft-tissue alterations using the Wilson method (about 15 transverse sections of each fetus).

There were no evident clinical signs of toxicity in dams treated at 10 or 30 mg/kg bw per day. Higher doses resulted in abortion (at 60 and 100 mg/kg bw per day) and signs of toxicity-like tremor and gasping breathing after tactile stimulation, diarrhoea and atactic gait. Mortality was observed at 60 mg/kg bw per day in one dam and in 13 dams at the highest dose. The body-weight gain was similar to that of controls at 10 and 30 mg/kg bw per day and was dose-related, decreasing at 60 and 100 mg/kg bw per day. In animals at 300 mg/kg bw per day, there was no body-weight gain, while animals at 1000 or 3000 mg/kg bw per day lost weight during the dosing period. At necropsy, dams at 300 mg/kg bw per and above were found to have dark brown colouration of the liver and kidneys, while severe dilatation of the duodenum and jejunum was observed at 1000 mg/kg bw per and above.

In the untreated control group, four fetuses from three litters had malformations of the spine, while in the vehicle control group, nine fetuses from eight litters had malformations affecting the spine, ribs and head (Table 2).

Table 2. Selected findings from a study of prenatal developmental toxicity in rats

Finding	Dose (mg/kg bw per day)								
	0 (un- treated)	0 (vehicle)	10	30	60	100	300	1000	3000
Total No. of animals	29	54	26	22	24	22	31	30	29
No. of pregnant animals	27	51	23	21	23	15	26	25	16
No. of dead animals	0	0	0	0	1	0	0	0	13
No. of abortions	0	0	0	0	2	3	0	0	0
Body weight (g):									
Day 6	235.6	225.6	221.6	213.7	216.9	210.0	231.2	227.7	220.5
Day 15	266.2	254.4	248.6	238.8	237.3	224.3	231.1	192.9	164.3
Body-weight gain (g), days 0–15	52.1	49.3	47.1	47.7	39.5**	37.3**	18.7**	–17.3**	–41.3**
No. of implantations/dam	11.93	11.08	11.00	9.33	11.48	10.07	10.88	11.88	9.81
Postimplantation loss (%)	4.94	4.25	3.86	6.12	50.76**	85.43**	100**	100**	100**
No. of live fetuses/dam	11.44	10.61	10.61	8.76	5.04	0.27	0	0	0
Mean fetal weight (g)	3.60	3.75**	3.54**	2.97**	2.36**	1.70**	—	—	—
No. of live fetuses	309	541	244	184	116	4	0	0	0
No. of malformed fetuses	4	9	1	77	104	4	—	—	—
Litters affected	3	8	1	19	18	3	—	—	—
Malformed fetuses (%)	1.29	1.66	0.41	41.85	89.66	100	—	—	—

From Hofmann & Peh (1987a)

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

At 10 mg/kg bw per day, only one fetus had a malformation of the spine; there was no increase in variations or retardations. Although the mean fetal weight was statistically significantly lower than in controls, there were no runts in this group.

At 30 mg/kg bw per day, there was a significant decrease in mean fetal weight, and one out of three of the fetuses were runts. Seventy-seven (42%) of the fetuses in 19 out of 21 litters had malformations affecting the spine, ribs, head (internal hydrocephalus) and sternum. There was also some increase in variations and delays.

At 60 mg/kg bw per day, 2 out of 23 animals aborted and 51% of the implantations in the remaining dams were dead. The mean fetal weight was significantly lower than in controls, and virtually all the fetuses were runts. Malformations were seen in 104 (90%) of fetuses from 18 litters, and there was also an increase in variations and retardations.

At 100 mg/kg bw per day, 15 pregnant animals produced only four live fetuses in three litters. All fetuses were runts and had malformations of the spine, ribs, limbs, heart, lungs and head. At 300, 1000, and 3000 mg/kg bw per day, all the embryos died in the early and intermediate phases of gestation.

The NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of clinical signs and reduced body-weight gain at 60 mg/kg bw per day and above. The NOAEL for developmental toxicity and teratogenicity was 10 mg/kg bw per day on the basis of reduced fetal weight and an increased incidence of malformations at 30 mg/kg bw per day (Hofmann & Peh, 1987a).

In a follow-up study of prenatal developmental toxicity intended to identify a NOAEL for malformations, particularly internal hydrocephalus, carbendazim (purity not indicated, technical active substance) was administered at daily doses 10 and 30 mg/kg bw per day by oral gavage in a 0.5% aqueous suspension of carboxymethylcellulose to groups of 29 or 30 Sprague-Dawley rats on days 6–15 of gestation, while two control groups of 20 animals were untreated or received the vehicle. The study was conducted in compliance with 1966 FDA guidelines. The dams were killed on day 20 of gestation, and the reproductive tract was examined for corpora lutea, implantation and resorption sites and live and dead fetuses. The fetuses were weighed, sexed and examined for external abnormalities. Two thirds of the live fetuses from each litter were selected at random and examined for skeletal alterations using a modification of the Dawson method, the remaining part of each litter was prepared and examined for soft-tissue alterations using the Wilson method (about 20 transverse sections of each fetus). In addition, the heads of all the fetuses were examined using the Wilson method (about eight transverse sections of each head).

There was no maternal toxicity apparent in this study. The mean number of implants, the resorption rate and the number of live fetuses did not show significant changes attributable to the test substance.

In the untreated control group, four fetuses from four litters had malformations (head, ribs and spine), while in the vehicle control group, four fetuses from three litters had malformations (ribs and spine). At 10 mg/kg bw per day, two fetuses from two litters had malformations (head and spine); there was no increase in variations or delays (Table 3).

At 30 mg/kg bw per day, mean fetal weight was significantly decreased, and the number of runts was increased. Eighty-one (23%) of the fetuses from 22 litters had malformations affecting the head, the spine and the ribs. Internal hydrocephalus was found in 17 (4.8%) of the fetuses, while 60 or 8 of the fetuses (16.8% or 2.2%) had cleft thoracic or lumbar vertebrae, respectively. In addition, there was an increase in variations and retardations in the group receiving the highest dose (mainly aplasia and/or displacement of individual sternbrae, dilatation of lateral ventricles in the brain).

The NOAEL for maternal toxicity was 30 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity and teratogenicity was 10 mg/kg bw per day on the basis of reduced fetal body weight and increased incidence of malformations and variations at 30 mg/kg bw per day (Hofmann & Peh, 1987b).

Table 3. Selected findings from a study of prenatal developmental toxicity in rats

Finding	Dose (mg/kg bw per day)			
	0 (untreated)	0 (vehicle)	10	30
No. of pregnant animals	20	20	29	30
Body weight (g):				
Day 6	227.4	223.9	230.3	226.6
Day 15	263.1	252.6	263.9	257.9
No of implantations/dam	13.60	12.00	12.38	12.60
Postimplantation loss (%)	2.21	4.17	3.62	5.29
No. of live fetuses	266	230	346	358
Mean fetal weight (g)	3.39	3.47**	3.57**	3.13**
No. of runts	0	2	0	29
No. of malformed fetuses / % of live fetuses	4/1.50	4/1.74	2/0.58	81**/22.63
No. of litters with malformed fetuses	4	3	2	22
Malformations:				
Internal hydrocephalus	2	0	1	17
Short ribs	0	0	0	1
Wavy ribs bilateral/unilateral	0/1	2/1	0/0	2/4
Cleft thoracic/lumbar vertebrae	1/0	1/0	1/0	60/8
Variations:				
Aplasia of individual sternebrae ^a	12	10	12	34
Displacement of individual sternebrae	2	1	2	19
Dilatation of the lateral ventricles	1	1	10	12
Dilatation of recess of 4th ventricle/sylvius aqueduct	0	1	2	5

From Hofmann & Peh (1987b)

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

^a Aplasia = negative results after staining with alizarin red.

In a study of prenatal developmental toxicity, groups of 25 Crl:CD BR rats were given carbendazim (purity, 98.8%) at a dose of 0, 5, 10, 20 or 90 mg/kg bw per day by gavage in a 0.5% aqueous suspension of carboxymethylcellulose on days 7–16 of gestation. The study complied with the principles of GLP and OECD test guideline 414. The dams were killed on day 22 of gestation, and the reproductive tract was examined for corpora lutea, implantation and resorption sites and live and dead fetuses. The fetuses were weighed, sexed and examined for external abnormalities. For each litter, the maximum stunted weight was calculated by subtracting the lightest weight from the total weight, dividing by the remaining number of fetuses and multiplying by 0.666. A fetus weighing the same or less than the maximum stunted weight was considered to be stunted; its weight was omitted when the mean litter weight was calculated. The first live fetus and thereafter every other fetus in each litter was decapitated and examined for visceral alterations and the sex verified. The heads were fixed in Bouin' fluid and examined. All stunted and externally malformed fetuses were also examined for visceral alterations; a decision to do a head examination was made on an individual basis. All remaining fetuses were stained skeletally with alizarin red S and examined for skeletal alterations.

Maternal toxicity was seen only at 90 mg/kg bw per day, in the form of depressed weight gain during the later part of the dosing period (days 13–17) and for days 17–22 of gestation. In

addition, mean feed consumption was depressed during the first 2 days of dosing, and mean liver weights and liver-to-body weight ratios were increased at the highest dose.

At 90 mg/kg bw per day, the low number of dams delivering pups (15 versus 24 for the control group) was attributed to the lower pregnancy rate (19 of 25 dams), one death (by mechanical dosing trauma) and three dams that had total resorptions. The increased mortality in utero at this dose was mainly caused by an increased incidence of early resorptions. Associated with these changes was a significant decrease in litter size (live fetuses per dam), with only the reduction in females per litter being statistically significant. No effect on survival was seen at the other doses (Table 4).

At doses of 20 and 90 mg/kg bw per day, embryo- and fetal toxicity was evident as a significant reduction in mean fetal weight and a significant increase in the mean percentage of fetuses per litter with variations. The increase in developmental variations that were described as being not related to retarded growth was for the most part caused by increases in skeletal variations (misaligned sternbrae and extra ossification sites of the ribs) and lesser due to visceral variations. The increase in variations that might be attributed to retarded development was primarily caused by increases in skeletal variations (vertebra showing bipartite ossification and dumb-bell centrum) (Table 5).

A significant increase in the incidence of malformations was seen in the highest dose group. These malformations included a variety of conditions mainly in the head (exencephaly, domed head, hydrocephaly), eyes (anophthalmia, microphthalmia), paws (clubbed) and skeletal malformations (fused vertebrae, ribs and sternum; hemivertebrae; rib hypoplasia; malformed scapula). Some of these malformations were also seen in the three fetuses affected at 20 mg/kg bw per day, but not at lower doses or in the controls. Thus, 20 mg/kg bw per day may be considered to be a threshold for malformations.

The NOAEL for maternal toxicity was 20 mg/kg bw per day on the basis of decreased body-weight gain and increased liver weight at 90 mg/kg bw per day. The NOAEL for developmental toxicity and teratogenicity was 10 mg/kg bw per day on the basis of decreased fetal body weight and increases in skeletal variations and malformations at 20 mg/kg bw per day and above (Alvarez, 1987).

Table 4. Selected maternal and reproductive findings from a study of prenatal developmental toxicity in rats

	Dose (mg/kg bw per day)				
	0	5	10	20	90
Body-weight gain (g), days 7–17/days 17–22	53.3/77.0	55.9/81.7	52.9/75.2	55.5/79.4	48.7/53.4*
Liver weight (g) / (%)	15.5/5.0	15.8/5.0	16.6/5.2	16.8/5.2	17.1*/5.3
No. of dams mated/pregnant	25/24	25/23	25/24	25/22	25/19*
No. of corpora lutea per dam	14.8	15.2	15.0	15.5	15.3
No of nidations per dam	13.3	13.7	12.6	13.5	13.5
No. of total litter resorptions	0	0	0	0	3
No. of resorptions per dam	0.3	0.2	0.9	0.5	3.5**
No. of live fetuses per dam	13.0	13.5	11.7	13.1	9.9*
Ratio of females/males	7.1/5.9	7.8/5.7	6.4/5.3	7.0/6.0	4.9*/5.0
Mean fetal weight (g), females/males	5.17/5.42	5.18/5.52	5.06/5.44	4.90*/5.12*	3.67**/3.98*

From Alvarez (1987)

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

Table 5. Selected fetal findings from a study of prenatal developmental toxicity in rats

Finding	Dose (mg/kg bw per day)				
	0	5	10	20	90
<i>External examinations</i> (fetuses/litters)	312/24	310/24	281/24	288/22	149/15
Malformations (fetuses/litters):	1/1	0	0	1/1	43/8**
Abdomen—gastroschisis	1/1	0	0	0	1/1
Eye bulge—none	0	0	0	0	12/6
—small	0	0	0	0	4/2
Head—domed	0	0	0	0	9/4
—exencephaly	0	0	0	0	17/2
Paw—clubbed	0	0	0	1/1	11/6
<i>Head examination</i> (fetuses/litters)	164/24	162/23	148/24	147/22	84/15
Malformations (fetuses/litters)	2/2	0	1/1	1/1	41/14**
Brain—hydrocephalus	0	0	0	0	24/10
—lateral ventricle distended	0	0	1/1	0	4/4
Eye—anophthalmia	2/2	0	0	0	21/10
—microphthalmia	0	0	0	1/1	4/3
<i>Skeletal examination</i> (fetuses/litters)	312/24	310/23	281/24	288/22	149/15
Malformations (fetuses/litters)	0	0	0	2/2	80/12**
Mean % fetuses affected per litter	0	0	0	0.7	59.2
Skull—fused	0	0	0	0	3/1
—domed frontal area	0	0	0	0	1/1
Vertebra—fused	0	0	0	0	77/11
—hemivertebra	0	0	0	0	23/10
—hypoplasia, atlas	0	0	0	0	2/1
—agenesis	0	0	0	1/1	4/3
Rib—fused	0	0	0	0	40/10
—hypoplasia	0	0	0	0	10/5
—none	0	0	0	1/1	4/2
—spur at angle	0	0	0	0	1/1
Sternum—fused sternebrae	0	0	0	1/1	12/6
—scrambled sternebrae	0	0	0	1/1	1/1
—cleft	0	0	0	0	1/1
Scapula—malformed	0	0	0	0	28/6
Developmental variations ^a (fetuses/litters)	12/7	12/9	12/8	29/14*	12/5
Mean % affected per litter	3.5	4.1	4.4	11.2	11.5
Ribs—extra ossification site, ce7	3/3	1/1	2/2	12/6	3/1
Sternum—1 misaligned sternebra	3/3	5/5	0	10/7	2/2
—≥ 2 misaligned sternebrae	0	1/1	1/1	2/2	3/2
Variations-retardations ^b (fetuses/litters)	42/17	65/19	40/16	87/19	38/7
Mean % affected per litter	12.1	19.8	14.6	31.2	46.9
Vertebrae—bipartite ossification	1/1	6/5	1/1	21/8	24/7
—dumb-belled centrum	7/5	16/8	7/6	44/13	26/7
<i>Malformations, total</i> (fetuses/litters)	3/2	1/1	1/1	3/3	91/15**
Mean % fetuses affected per litter	1.3	0.3	4.2	1.0	69.9**

Developmental variations, total (fetuses/litters)	22/14	20/12	19/12	42/16	19/5
Mean % fetuses affected per litter	10.8	6.6	6.8	16.1*	18.2*
Variations-retardations, total (fetuses/litters)	43/18	66/19	40/16	87/19	38/7
Mean % fetuses affected per litter	12.4	20.1	14.6	31.2*	46.9**
<i>Variations, total</i> (fetuses/litters)	64/23	80/20	54/19	115/21	44/7
Mean % fetuses affected per litter	22.9	25.0	19.5	41.6**	52.5**

From Alvarez (1987)

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

^a Developmental variations: mainly ribs with extra ossification sites and misaligned sternebrae.

^b Variations due to retardation: mainly bipartite ossification and dumb-belled centrum of vertebrae; reduced ossification of vertebrae, skull and ribs.

Rabbits

In a study of prenatal developmental toxicity, groups of 20 artificially inseminated New Zealand White [Hra:(NZW)SPF] rabbits were given carbendazim (purity, 98.7%) at a dose of 0, 10, 20, or 125 mg/kg bw per day by gavage in aqueous 0.5% carboxymethylcellulose on days 7–19 of presumed gestation. The study was conducted in compliance with the principles of GLP and according to OECD test guideline 414. The dams were killed on day 29 of gestation, and the reproductive tract was examined for corpora lutea, implantation and resorption sites and live and dead fetuses. The fetuses were weighed, sexed and examined for external abnormalities and for visceral alterations. The brain was free-hand transverse-sectioned (a single cut at the level of the anterior fontanelle) and examined. The bodies of all fetuses obtained after a minimum of 27 days of gestation were processed, stained with alizarin red S and evaluated for skeletal alterations.

Two rabbits in the group receiving the highest dose aborted, one on day 22 and the other on day 25 of gestation. As this incidence is slightly in excess of the incidence in historical controls, the effect is considered to be treatment-related. Maternal weight gain and feed consumption were reduced at 125 mg/kg bw per day during the dosage period, and significantly increased after the dosage period.

The incidence of pregnancy was similar at all doses. A slight decrease in the number of corpora lutea was observed for the group receiving the highest dose. Treatment at 20 and 125 mg/kg bw per day resulted in decreased implantation, increased resorption and decreased live litter size (Table 6). Additionally, at the highest dose, fetal body weight was lower than that seen in the controls, but the effect was not statistically significant. The average percentage of malformed fetuses per litter was significantly increased at 125 mg/kg bw per day. Compound-related malformations at the highest dose consisted of malformed cervical vertebrae and interrelated malformations of the ribs and proximate thoracic vertebrae.

The NOAEL for maternal toxicity was 20 mg/kg bw per day on the basis of a slight increase in abortions and decreased body-weight gain and feed consumption at 125 mg/kg bw per day. The NOAEL for developmental toxicity was 10 mg/kg bw per day on the basis of decreased implantation, increased resorption and decreased live litter size at ≥ 20 mg/kg bw per day, and decreased fetal weights and an increased incidence of fetuses with malformations at 125 mg/kg bw per day (Christian et al., 1985).

1.3 Additional studies

Studies on the effects of carbendazim on spermatogenesis, including a newly submitted review (Hurt, 1993), and newly submitted studies on genotoxicity were evaluated as part of this monograph addendum.

Table 6. Selected findings from a study of prenatal developmental toxicity in rabbits

	Dose (mg/kg bw per day)			
	0	10	20	125
Body-weight gain (kg):				
Days 7–10	+0.02	+0.01	0.00	−0.05*
Days 13–16	+0.06	+0.05	+0.06	−0.05*
Days 24–29	−0.11	−0.06	−0.05	+0.05**
Feed consumption (g/kg bw):				
Days 7–10	37.5	37.0	37.4	34.0
Days 13–16	31.5	32.6	33.3	25.8
Days 24–29	13.6	14.8	16.2	32.7**
No. of animals pregnant/No. inseminated	16/20	17/20	17/20	18/20
No. of animals aborted	0	0	0	2
No. of animals delivered naturally	2	1	0	0
No. of litters ^a	14	16	16	9
Mean No. of corpora lutea ^b	10.7	11.3	10.5	8.9*
Mean No. of implantations ^b	7.7	7.4	6.0*	5.9*
Mean No. of resorptions ^b	0.2	1.0	0.6	2.9**
Mean % of resorptions ^b	4.0	8.4	15.3	52.3**
No. of litters with total resorptions	0	0	1	7**
No. of litters with > 2 resorptions	0	2	1	7
No. of live fetuses ^a	105	103	91	49
Mean no. of live fetuses per litter ^a	7.5	6.4	5.7*	5.4*
Mean fetal weight (g) ^a	43.32	44.10	42.15	40.83
No. of litters evaluated for developmental effects	15	17	16	9
Litters of fetuses with any alteration/malformation	11/9	8/7	9/5	8/8
Fetuses with any alteration/malformation	19/11	20/12	18/6	25/24
% Fetuses with any alteration/malformation per litter	19.4/10.0	18.1/10.1	18.2/8.9	54.3/52.7**
Cervical vertebrae—hemivertebra ^c	0	0	0	3/2
—asymmetric ^c	0	0	0	8/2
—fused ^c	0	0	0	6/2
—bifid ^c	0	0	0	3/1
Thoracic vertebrae—bifid ^c	0	0	0	3/2
Ribs—fused ^c	1/1	0	0	6/4

From Christian et al. (1985)

* $p \leq 0.05$; ** $p \leq 0.01$ ^a Excludes rabbits that aborted, delivered or were not pregnant, or had only resorbed conceptuses in utero.^b Excludes rabbits that aborted, delivered or were not pregnant; includes those with total resorption.^c No. of fetuses/No. of litters

(a) *Effects on spermatogenesis*

In a special study on testicular effects, groups of 20 male rats aged 97–105 days were given carbendazim as a single oral dose at 0, 50, 100, 200, 400 or 800 mg/kg bw (purity not indicated) and were killed 2 days (8 animals/dose) or 70 days (12 animals/dose) after treatment.

On day 2, at 50 mg/kg bw, round spermatids were sloughed (prematurely released) from stage I and II epithelium and elongated spermatids were sloughed from stage VII epithelium; however, the percentage of seminiferous tubules exhibiting epithelial sloughing was only marginally increased (< 10%) and not statistically significantly different from the controls. At 100 mg/kg bw, the disappearance of germ cells was more severe and sloughing of elongated spermatids extended into stages XII through XIV. In animals treated at 100 mg/kg bw or more, a statistically significant and dose-dependent increase in testicular weight was seen that was accompanied by significant increases in mean seminiferous tubular diameter at 400 and 800 mg/kg bw. In addition, at doses of ≥ 100 mg/kg bw, there was a dose-dependent increased incidence of occlusions in the efferent ductules of the testes. The rete testis was swollen with sloughed germ cells, indicating that ductal blockage had occurred further down the tract; 50% or more of the efferent ductules were occluded. The occlusions were characterized as compacted luminal contents, spermatid granulomas, mineralizations, and obliteration of the original lumen by fibrotic connective tissue. At doses of ≥ 200 mg/kg bw, missing germ cells extended into all stages except stages IX–XI, while at doses of 400 and 800 mg/kg bw, some seminiferous epithelia were damaged so severely that it was difficult to identify the stage.

On day 70, mean seminiferous tubule diameter was statistically significantly decreased at all doses in a dose-dependent relationship. Histologically, these decreases were associated with a dose-dependent increase in seminiferous tubular atrophy (statistically significant at ≥ 100 mg/kg bw). No atrophic tubules were seen in the control rats, however, atrophy of a few seminiferous tubules in one testicle was noted at 50 mg/kg bw. The atrophied tubules contained primarily Sertoli cells and occasional spermatogonia and were surrounded by a thickened basement membrane. Pathological alterations were also noted in the efferent ductules of the treated animals, 50% or more of the ducts being occluded in rats dosed at ≥ 100 mg/kg bw. Minimal effects were seen at 50 mg/kg bw; slight abnormal growth of the efferent ductules was seen in only one specimen. The occlusions were characterized as compacted luminal contents, spermatid granulomas, mineralizations and obliterations of the original lumen by fibrotic connective tissue. In addition, mean testis weight showed a dose-dependent decrease that was statistically significant at doses of 100 mg/kg bw and above.

Overall, the NOAEL for effects on spermatogenesis was 50 mg/kg bw, since the minimal changes observed at this dose were considered to be of equivocal toxicological relevance (Nakai et al., 1992).

In a special study on effects on spermatogenesis by Hilscher et al. (1992), male Wistar rats were fed diets containing carbendazim (purity, 99.4%) at a concentration of 0, 10, 70, or 500 ppm for 182 days. A number of testicular end-points were examined: weight, histology, seminiferous tubular area, percent interstitial tissue, degeneration index of meiotic divisions, nuclear areas of round spermatids, enzyme histochemistry (thiamine pyrophosphatase, alanine aminotransferase, alkaline phosphatase, Ca-ATPase, Mg-ATPase, and various steroid dehydrogenases), and autoradiographic measures (nuclear area, silver grain count and density of various germ cells). A mating phase was also included after 182 days of treatment. There were no effects on fertility parameters, testicular weight, the area of seminiferous tubules or interstitial tissue, or epididymal structures and enzyme activities. However, for parameters that were less understood and generally regarded as research probes, there were three end-points (degeneration index of meiotic divisions, and the nuclear area and silver grain count of preleptotene spermatocytes) that did reveal statistically significant differences between control and treated groups. Evaluation of these parameters had methodological and test design deficiencies, which confounded interpretation of the results. Also, the study author concluded that the dose-related increases in the values for

nuclear areas and silver grain counts in preleptotene spermatocytes in the treated groups were of no major significance. Therefore, the results of the study support no biologically significant effects of carbendazim on spermatogenesis in rats at dietary concentrations of up to 500 ppm, equal to about 29–43 mg/kg bw per day (Hurt, 1993).

(b) *Genotoxicity*

The mechanism of action for carbendazim is well defined: the substance exerts its biological activity by binding to tubulin protein, disrupting microtubule assembly, preventing the formation of spindles at cell division, and thus resulting in the mal-segregation of chromosomes during cell division. It has been shown repeatedly that carbendazim does not interact directly with DNA and has given negative results in tests for gene mutations, structural chromosome aberrations (clastogenesis), and DNA damage and repair in a variety of test systems in vitro and in vivo, including studies in mammalian somatic and germ cells. However, consistent with its mode of action, carbendazim induces numerical chromosome aberrations (aneuploidy) in mammalian and non-mammalian cell systems in vitro and in mammalian somatic and germ cells in vivo.

Table 7 summarizes the results of newly submitted unpublished studies and relevant publications that included sufficient experimental detail and data.

(i) *In vitro*

In order to demonstrate the existence of a threshold level for the induction of aneuploidy by carbendazim (purity, 99.7%), a modified test for micronucleus formation in vitro was used coupled with fluorescence in-situ hybridization (FISH) to determine the distribution of chromosomes between daughter nuclei in cytokinesis-blocked binucleated human lymphocytes. Slide preparations were hybridized with centromeric DNA probes specific for six different human chromosomes: 1 and 8 (trisomies associated with a number of tumour types), 11 (associated with Wilm's tumour), 17 (associated with *p53* gene and breast cancer predisposition), and X and 18 (frequently associated with live aneuploidies). One thousand cells on two replicate slides were evaluated; abnormalities in chromosome distribution were classified as chromosome loss, chromosome gain, non-disjunction, or polyploidy. The presence of micronuclei carrying a centromeric signal was also recorded. The threshold was defined as the lowest concentration that produced a statistically significant increase in aneuploidy. At low concentrations, the frequencies of non-disjunction, chromosome loss, and chromosome gain were similar to control levels until specific points within the dosing range when concentration-dependent increases occurred for each end-point. Non-disjunction occurred at higher frequencies and generally at lower concentrations than for chromosome loss and chromosome gain and was considered to be the most sensitive aneugenic event for determining a threshold. For each end-point, the shapes of the dose–response curves for the six chromosomes were nearly identical. Threshold concentrations for non-disjunction caused by carbendazim were 600 ng/ml for chromosomes 17 and X, 700 ng/ml for chromosome 1, and 800 ng/ml for chromosomes 8, 11, and 18 (Marshall, 1996a; Bentley et al., 2000).

A threshold mechanism of action was also reported for carbendazim (purity not reported) and three other well-known mitotic spindle poisons (colchicine, mebendazole, and nocodazole) using a similar experimental test system in vitro with cultured human lymphocytes, as in Marshall (1996a). Chromosome loss was analysed by identifying micronuclei with centromeres using a fluorescent human pancentromeric DNA probe. Non-disjunction was evaluated in cytokinesis-blocked binucleated lymphocytes in combination with centromeric probes for chromosomes 1 and 17. For both end-points, similarly shaped dose–response curves were obtained for the four mitotic spindle inhibitors. In each case, the first concentration at which a statistically significant increase occurred was greater for chromosome loss than for non-disjunction. For carbendazim, the

threshold concentration for non-disjunction was 1.05 $\mu\text{mol/l}$, corresponding to about 200 ng/ml (Elhajouji et al., 1995, 1997).

The meiotic maturation in vitro of cultured mouse (CF-1, Harlan Sprague-Dawley) oocytes was evaluated after exposure to carbendazim (purity, 99.7%) at a concentration of 0, 3, 10, or 30 $\mu\text{mol/l}$ (corresponding to 0, 600, 2000, and 6000 ng/ml). Exposures were for a period of 6–8 h during initial stages of in-vitro maturation and meiotic metaphase I, during a 8–9 h interval between metaphase I and metaphase II, or 14–16 h during the entire period of maturation. Meiotic maturation was assessed in respect to chromosome organization, meiotic spindle microtubules, and cortical actin using fluorescent labelling for each of the structures. Exposure to carbendazim resulted in a dose-dependent inhibition of cell cycle progression at meiotic metaphase I, but did not interfere with progress to metaphase II except at the highest concentration. A loss in nonacetylated microtubules and a decrease in spindle size was noted at 3 and 10 $\mu\text{mol/l}$. At a concentration of 30 $\mu\text{mol/l}$, spindle assembly was prevented when carbendazim was added at the beginning of meiotic maturation or spindle pole disruption and fragmentation were caused when carbendazim was added to preformed spindles. Dispersed chromosomes were retained in the metaphase-plate location. Polar body extrusion was also impaired, and abnormal polar bodies were observed in most treated oocytes. The results of this study suggested that carbendazim disrupts cell cycle progress in oocytes by altering meiotic spindle microtubule stability and spindle pole integrity (Can & Albertini, 1997).

(ii) *In vivo: studies in somatic cells*

In a study conducted to confirm that the carbendazim-induced formation of micronuclei in bone marrow polychromatic erythrocytes (PCEs) was caused by aneuploidy rather than by structural chromosome (clastogenic) damage, groups of five male and five female BDF1 mice were given carbendazim (purity, 99.3%) at a dose of 0, 66, 1646, or 3293 mg/kg bw in 0.5% aqueous methylcellulose by oral intubation, and the mice were sacrificed after approximately 48 h. Doses were selected according to the results of a preliminary experiment where severe bone-marrow toxicity, as indicated by decreased proportions of PCEs/1000 erythrocytes, was observed at 3293 and 5000 mg/kg bw. Cyclophosphamide and vincristine sulfate were used as positive clastogenic and aneugenic controls, respectively. Using a specialized staining technique with immunofluorescent antikinetochore antibodies, micronuclei were assessed for the presence or absence of centromeres or centromere-associated proteins (kinetochores). Kinetochore positive (KC+) micronuclei were presumed to contain intact chromosomes and were indicative of aneuploidy; kinetochore-negative (KC-) micronuclei were considered to be acentric chromosome fragments resulting from clastogenic damage. Classification as an aneugen or clastogen was based on the ability to induce high proportions of KC+ or KC- micronuclei, respectively. Carbendazim induced statistically significant increases in the frequencies of total micronucleated PCEs (MN-PCEs) and KC+ MN-PCEs in female mice at 1646 and 3293 mg/kg bw and in male mice at 3293 mg/kg bw. Statistically significant dose-related trends were also observed in both sexes for these two parameters. Greater than 80% of the total MN-PCEs contained kinetochores. The frequency of KC- MN-PCEs was significantly increased in female mice at 3293 mg/kg bw; however, this increase was not considered to be evidence for clastogenicity since similar increases in KC- MN-PCEs were detected in male and female mice given the aneugenic positive control vincristine sulfate. Carbendazim also induced statistically significant decreases in the proportion of PCEs/1000 erythrocytes in females at doses of 1646 and 3293 mg/kg bw; significant dose-related decreases were observed in males and females. At 66 mg/kg bw, the frequencies of total MN-PCEs, KC+ MN-PCEs and KC- MN-PCEs, and the proportion of PCEs were similar to values for these parameters in the controls (Bentley, 1992; Sarraf et al., 1994).

Table 7. Results of additional studies of genotoxicity with carbendazim

End-point	Test object	Concentration	Purity (%)	Result	Reference
<i>In vitro</i>					
Aneuploidy induction ^{a, b}	Human lymphocytes	(a) 0–5000 ng/ml (b) 0–2000 ng/ml	99.7	Positive (≥ 600 ng/ml) (NOEL=500 ng/ml)	Marshall (1996); Bentley et al. (2000)
Aneuploidy induction ^a	Human lymphocytes	0–10 µmol/l (about 0–2000 ng/ml)	NR	Positive (≥ 200 ng/ml)	Elhajouji et al. (1995, 1997)
Disruption of meiotic cell cycle	CF-1 mouse oocytes	0–30 µmol/l (about 0–6000 ng/ml)	99.7	Positive (≥ 600 ng/ml)	Can & Albertini (1997)
<i>In vivo</i>					
Micronucleus formation	NMRI mouse bone marrow	2 × 50, 500 or 5000 mg/kg bw orally, 24 h apart	NR	Positive (≥ 500 mg/kg bw)	Mayer & Kramer (1980)
Micronucleus formation	NMRI mouse bone marrow	2 × 50, 500 or 5000 mg/kg bw orally, 24 h interval	NR	Positive (≥ 500 mg/kg bw)	Mayer et al. (1980)
Micronucleus formation	NMRI mouse bone marrow	1 × 50 or 200 mg/kg bw orally	99.4	Negative	Müller (1990)
Micronucleus formation ^b	B6D2F1/Cr-1BR mouse bone marrow	1 × 66, 1646 or 3293 mg/kg bw orally	99.3	Positive ^d (≥ 1646 mg/kg bw)	Bentley (1992); Sarraf et al. (1994)
Micronucleus formation	Wistar rat bone marrow	1 × or 2 × 150 mg/kg bw orally	NR	Positive (≥ 150 mg/kg bw)	Ashby & Tinwell (2001)
Micronucleus formation	Swiss mouse colon epithelial cells	1 × 500 or 1000 mg/kg bw orally	97	Positive (≥ 500 mg/kg bw)	Vanhouwaert et al. (2001)
Aneuploidy induction ^c	Syrian hamster oocytes	1 × 1000 mg/kg bw orally	95	Positive	Jeffay et al. (1996)
Micronucleus formation ^b	Sprague-Dawley rat spermatids	1 × 50, 100 or 400 mg/kg bw orally	NR	Positive ^d (≥ 100 mg/kg bw)	Matsuo et al. (1999)
Aneuploidy induction ^a	Wistar rat sperm	1 × 50, 150, 450 or 800 mg/kg bw orally	NR	Positive ^e (≥ 150 mg/kg bw)	de Stoppelaar et al. (1999)
Aneuploidy induction ^a	(102/ElxC3H/El)F ₁ mouse sperm	1 × 20, 50, 150 or 500 mg/kg bw orally	99.1	Negative	Adler (2001)
Dominant lethal mutation	NMRI mouse	1 × 1280 mg/kg bw intraperitoneally	94%	Negative	Hofmann & Peh (1973)

^a Test system using fluorescence in-situ hybridization (FISH) to detect aneuploidy and/or polyploidy^b Test with staining for centromeres or centromere-associated proteins (kinetochores)^c Cytogenetic analysis of chromosome number^d Increased frequency of kinetochore-positive micronuclei^e Increased frequency of total hyperhaploid sperm

NR, not reported

In response to a study reported later (de Stoppelaar et al., 1999), the ability of carbendazim to induce micronucleus formation in the bone marrow of rats was subsequently evaluated as earlier studies had demonstrated the activity of this substance only in assays for micronucleus formation in mouse bone marrow after one or two doses. Wistar-derived rats received carbendazim (source and purity not reported) as either one or two oral doses at 150 mg/kg bw suspended in corn oil; concurrent controls were given corn oil alone. All animals were terminated 24 h after the last dose, and 2000 polychromatic erythrocytes were evaluated from bone-marrow smears. Carbendazim induced a statistically significant and reproducible increase in the incidence of micronuclei, confirming the induction of aneuploidy in the somatic cells of the rat bone marrow (Ashby & Tinwell, 2001).

In a novel cytogenetics assay *in vivo* in which the induction of micronuclei was assessed in epithelial cells of the colon for several mutagenic substances, SPF albino Swiss mice were given carbendazim (purity, 97%) as a single oral dose at either 500 or 1000 mg/kg bw in Methocel-Tween. Animals were terminated at either 24 or 48 h after exposure. One thousand epithelial cells within the intestinal crypts were evaluated for the presence of micronuclei. Carbendazim appeared to induce a small but statistically significant increase in the frequency of micronuclei at both doses and sampling intervals, but a dose-response relationship was not observed. Although the authors stated that an increase of micronuclei did not occur in the accompanying evaluation of bone marrow, the data presented show a fivefold and threefold increase (not statistically significant) in micronucleated polychromatic erythrocytes at the 24 h sampling interval after carbendazim doses of 500 and 1000 mg/kg bw, respectively (Vanhauwaert et al., 2001).

(iii) *Studies in germ cells in vivo*

In a study conducted to assess the ability of carbendazim to induce aneuploidy in oocytes, female Syrian hamsters were given carbendazim (purity, 95%) as a single oral dose at 1000 mg/kg bw in corn oil on the afternoon of proestrus coinciding with meiotic maturation of the oocytes. In unfertilized oocytes, the treatment resulted in an apparent increase in aneuploidy frequency (37.2% compared with 13.5% in the control group) as indicated by cytogenetic analysis of the chromosome number. There was no evidence for any carbendazim-induced structural abnormalities. In animals that were allowed to mate after dosing, fertilization rate and number of oocytes recovered was not impaired. However, preimplantation embryonic development was affected as evidenced by an increase in the proportion of embryos that failed to reach the expected stages of development (e.g. eight-cell, morula, or blastocyst stages). In addition, the mean number of implantation sites was lowered (Jeffay et al., 1996).

In a study conducted to assess the ability of carbendazim to induce micronucleus formation in round (step I, immature) spermatids, groups of six male Sprague-Dawley rats were given carbendazim (source and purity not reported) as single oral doses at 0, 50, 100 or 400 mg/kg bw in corn oil. The animals were sacrificed 24 h after dosing, and following staining for DNA, 1500 spermatids per individual rat, were evaluated for the formation of micronuclei. A statistically significant increase was observed only in the group at receiving the intermediate dose; frequencies of micronuclei were nearly identical at 50 and 400 mg/kg bw, showing the absence of a dose-response relationship. The authors suspected that testicular damage at the highest dose may have been responsible for reduced frequencies of micronucleated spermatids. Immunocytochemistry of spermatids from the group receiving a dose of 100 mg/kg bw indicated that as much as 68% of the micronuclei contained kinetochores compared with 30% in the control group. The authors concluded that carbendazim-induced micronuclei in rat spermatids were caused by aneuploidy, rather than the clastogenic activity of the test compound (Matsuo et al., 1999).

In a study conducted to assess the ability of carbendazim to induce numerical chromosome aberrations in sperm and micronuclei in peripheral blood erythrocytes, groups of three to five

Wistar rats received carbendazim (purity not reported) as single oral doses at 0, 50, 150, 450, or 800 mg/kg bw in corn oil. Dual-colour FISH was performed on epididymal sperm obtained 31 days after treatment using probes for chromosomes 4 and Y. At least 10 000 sperm per animal were evaluated. Further categorization of hyperhaploid sperm (44Y, 4YY, 44) by nuclear size was conducted to evaluate which sperm were actually diploid. The authors reported that increases in hyperploid sperm were observed at doses of 150 mg/kg bw and greater and that, based on evaluation of nuclear size, the majority were diploid sperm. A subsequent experiment was conducted using tri-colour FISH using probes for two somatic chromosomes 4 and 19 and for chromosome Y. As noted by the authors, the three-chromosome analysis was considered to be a more accurate method for assessing aneuploidy and diploidy in sperm. In this second experiment, 11 doses of carbendazim were used ranging from 2.5 to 800 mg/kg bw; each group contained three animals. In addition, 2000 peripheral blood erythrocytes per animal were evaluated for the presence of micronuclei in blood samples taken 48 h after treatment. The results showed a preferential induction of diploid sperm by carbendazim, but only at the highest dose tested, 800 mg/kg bw, and the frequency was lower than that reported in the first experiment where nuclear size had been considered for classification of aneuploid and diploid sperm. No increases in micronuclei were observed in peripheral erythrocytes at any carbendazim dose. On the basis of this study, the authors suggested that diploidy could be induced in sperm at a lower dose than could micronucleus formation in peripheral blood erythrocytes. However, it should be noted that analysis of peripheral blood samples for micronuclei in this study was likely suboptimal at the selected sampling time as micronucleated erythrocytes may have been efficiently removed by the spleen in rats (de Stoppelaar et al., 1999).

In a study conducted to assess the ability of carbendazim to induce aneuploid and/or polyploid sperm, groups of five male mice (102/ElxC3H/El F1) were given carbendazim (purity, 99.1%) as a single dose at 0, 20, 50, 150, or 500 mg/kg bw by oral gavage in corn oil. Doses were selected based on previously reported aneuploidy in rodent sperm (de Stoppelaar et al., 1999) and a preliminary experiment in the testing laboratory that suggested there was an increase frequency of diploid sperm at 500 and 1000 mg/kg bw (Adler et al., 2002). The positive control, colchicine, was prepared in water and administered by intraperitoneal injection. Sperm were collected from the epididymis from each control and carbendazim-treated animal 22 days after dosing, while positive controls were sampled 24 days after dosing, owing to known delays in sperm maturation by treatment with colchicine. Abnormal chromosome numbers in sperm were analysed with fluorochrome-labelled DNA probes specific for chromosomes X, Y, and 8 by multi-colour FISH. At least 10 000 sperm per animal were evaluated and assigned phenotypes (normal haploid: X8 or Y8; hypohaploid: 08, X0, or Y0; hyperhaploid: XX8, YY8, XY8, X88 or Y88; and diploid: XY88, XX88, or YY88) according to the combination of fluorescent signals in each nucleus. Statistical analyses of the data were carried out using the individual animal as the experimental unit.

The Meeting noted that analysis of data generated in the earlier experiment by this laboratory (Adler et al., 2002) compared pooled incidences of abnormal sperm from all animals within a treatment group to that of the control group resulting in statistical significance. Re-analysis using the animal as the experimental unit failed to result in statistical significance. A dose-response relationship was also absent. No clinical signs of toxicity were observed after treatment at any dose. As expected, a statistically significant increase in the frequency of hyperhaploid sperm was observed in the positive control animals when compared with the negative control group. However, no statistically significant nor dose-related increases in the frequency of hypohaploid, hyperhaploid, or diploid sperm were observed in the groups treated with carbendazim. The incidence of diploidy, which had been previously reported by the laboratory to have been induced in an earlier experiment, was 0, 3, 4, 2, and 4 in approximately 50 000 sperm scored at 0, 20, 50, 150, and 500 mg/kg bw, respectively. The Meeting concluded that the NOAEL was 500 mg/kg bw (Adler, 2001).

Comments

Carbendazim has low acute toxicity: the oral LD₅₀ is > 10 000 mg/kg bw in rats. The clinical signs of toxicity after single high doses were generally nonspecific. Degenerative changes in the testes and epididymides were observed in rats given single oral doses at ≥ 1000 mg/kg bw.

In two short-term studies of toxicity in rats, the overall NOAEL was 2000 ppm (equal to 163 mg/kg bw per day) on the basis of reduced body weight and inhibition of spermatogenesis at 10 000 ppm (equal to 780 mg/kg bw per day) and above. In a 28-day dose range-finding study in dogs, the NOAEL was 500 ppm (equal to 19 mg/kg bw per day) on the basis of liver toxicity at 2500 ppm (equal to 96 mg/kg bw per day).

Carbendazim has been adequately tested in a range of assays for genotoxicity. Carbendazim causes changes in chromosome number (aneuploidy) both in vitro and in vivo (in somatic cells and germ cells) as a result of its interference with mitotic spindle proteins. The effects were seen in tests for the induction of micronuclei or aneuploidy in vivo after single high doses (100 mg/kg bw and above), with a NOAEL of 50 mg/kg bw. The mechanism by which aneuploidy is induced by carbendazim is well understood and consists of inhibition of the polymerization of tubulin, the protein that is essential for the segregation of the chromosomes during cell division. The nature of the mechanism is thus consistent with the identification of a dose that has no toxicological effect. Carbendazim does not cause gene mutations or structural chromosomal aberrations.

The Meeting concluded that the genotoxic effect of carbendazim is a threshold phenomenon.

In a study of developmental toxicity in rats given diets containing carbendazim, the NOAEL for both maternal and developmental toxicity was 10 000 ppm (equal to 747 mg/kg bw per day, the highest dose tested). There was no evidence for embryo- and fetotoxicity or teratogenicity after dietary administration of carbendazim.

Studies of developmental toxicity in rats and rabbits given carbendazim by oral gavage clearly demonstrated that carbendazim is a developmental toxicant and teratogen.

In three studies of developmental toxicity in rats treated by gavage, maternal toxicity (clinical signs, decreased body-weight gain, abortion) was observed at doses of 60 mg/kg bw per day and above. Developmental toxicity consisting of decreased fetal weights and an increased percentage of fetuses with variations per litter was seen at doses of 20 mg/kg bw per day and above. The increased incidence in variations was largely attributable to delayed development and thus correlated with the reduction in fetal weight. The incidence of malformations including hydrocephaly, anophthalmia, microphthalmia, axial skeletal malformations or malformed scapulae was significantly increased at doses of 30 mg/kg bw per day and above in two studies and at 90 mg/kg bw per day in one study, with a slightly higher incidence of skeletal malformations at 20 mg/kg bw per day than in controls. The threshold for embryo/fetotoxicity and teratogenicity was thus considered to be 20 mg/kg bw per day. For the three studies, the overall NOAEL for maternal toxicity was 30 mg/kg bw per day, while the overall NOAEL for developmental toxicity was 10 mg/kg bw per day.

In a study of developmental toxicity in rabbits treated by gavage, maternal toxicity (reduction of feed consumption and body-weight gain, abortion) was observed at 125 mg/kg bw per day, the highest dose tested. Treatment at 20 and 125 mg/kg bw per day resulted in decreased implantation, increased resorption and decreased size of live litters. Additional effects consisting of decreased fetal body weights and increased incidence of malformations of the cervical vertebrae, ribs and thoracic vertebrae were seen at 125 mg/kg bw per day. The NOAEL for maternal toxicity was 20 mg/kg bw per day and the NOAEL for developmental toxicity was 10 mg/kg bw per day.

In a study of toxicity to the male reproductive system in rats, significant testicular and efferent ductal alterations were seen 2 days after the administration of single doses at 100 mg/kg bw and above by gavage. The major cause of testicular atrophy observed at later times (70 days) after dosing was occlusion of the efferent ductules. The NOAEL was 50 mg/kg bw.

Toxicological evaluation

The Meeting established an ARfD of 0.1 mg/kg bw based on an overall NOAEL of 10 mg/kg bw per day for developmental toxicity from three studies in rats and one study in rabbits, and a safety factor of 100. The Meeting concluded that this ARfD applies only to women of childbearing age.

For the general population, including children, the Meeting established an ARfD of 0.5 mg/kg bw based on the NOAEL of 50 mg/kg bw in the study of toxicity to the male reproductive system in rats and supported by the studies on micronucleus or aneuploidy induction in vivo, using a safety factor of 100.

An additional safety factor for the severity of the effects was considered to be unnecessary, since the underlying mechanism is clearly understood and there is a clear threshold for these effects.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Developmental toxicity ^a	Maternal toxicity	30 mg/kg bw per day	60 mg/kg bw per day
		Developmental toxicity	10 mg/kg bw per day	20 mg/kg bw per day
	Acute toxicity, special study	Testicular effects	50 mg/kg bw	100 mg/kg bw
Rabbit	Developmental toxicity	Maternal toxicity	20 mg/kg bw per day	125 mg/kg bw per day
		Developmental toxicity	10 mg/kg bw per day	20 mg/kg bw per day

^a Three studies combined

Estimate of acute reference dose

0.1 mg/kg bw for women of childbearing age

0.5 mg/kg bw for the general population, including children

References

- Adler, I.-D. (2001) Mouse sperm-FISH assay with carbendazim. Unpublished report No. DuPont-4533 from GSF-National Research Centre for Environment and Health, Institute of Experimental Genetics, Neuherberg, Germany. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
- Adler, I.-D., Schmid, T.E. & Baumgartner, A. (2002) Induction of aneuploidy in male mouse germ cells detected by the sperm-FISH assay: A review of the present data base. *Mutat. Res.*, **504**, 173–182.
- Alvarez, L. (1987) Teratogenicity study of INE-965 (carbendazim) in rats. Unpublished report No MR-7976-001 HLR 281-87 from E.I. DuPont de Nemours and Co., Haskell Laboratory, Newark, Delaware, USA. Previously submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
- Ashby, J. & Tinwell, H. (2001) Continuing ability of the rodent bone marrow micronucleus assay to act as a predictor of possible germ cell mutagenicity of chemicals. *Mutat. Res.*, **478**, 211–213.
- Bentley, K.S. (1992) Classification of DPX-E965-299 (Carbendazim, MBC)-induced micronuclei in mouse bone marrow erythrocytes using immunofluorescent antikinetochore antibodies. Unpublished report

- HLR 569-92 from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
- Bentley, K.S., Kirkland, D., Murphy, M. & Marshall, R. (2000) Evaluation of thresholds for benomyl- and carbendazim-induced aneuploidy in cultured human lymphocytes using fluorescence *in situ* hybridization. *Mutat. Res.*, 464: 41–51.
- Can, A. & Albertini, D.F. (1997) Stage specific effects of carbendazim (MBC) on meiotic cell cycle progression in mouse oocytes. *Mol. Reprod. Dev.*, **46**, 351–362.
- Christian, N.S., Hoberman, A.M. & Feussner, E.L. (1985) Developmental toxicity study of carbendazim administered via gavage to New Zealand white rabbits. Unpublished report, study No. 104-008, from Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Previously submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
- Culik, R., Sherman, H. & Zapp, J.A. (1970) Teratogenic study in rats with 2-benzimidazole-carbamic acid, methyl ester (INE-965). Report No. HLR 466-70 from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
- de Stoppelaar, J.M., Van de Kuil, T., Bedaf, M., Verharen, H.W., Slob, W., Mohn, G.R., Hoebee, B. & Van Benthem, J. (1999) Increased frequencies of diploid sperm detected by multicolour FISH after treatment of rats with carbendazim without micronucleus induction in peripheral blood erythrocytes. *Mutagenesis*, **14**, 621–631.
- Elhajouji, A., Tibaldi, F. & Kirsch-Volders, M. (1997) Indication for thresholds of chromosome non-disjunction versus chromosome lagging induced by spindle inhibitors *in vitro* in human lymphocytes. *Mutagenesis*, **12**, 133–140.
- Elhajouji, A., van Hummelen, P. & Kirsch-Volders, M. (1995) Indications for a threshold of chemically-induced aneuploidy *in vitro* in human lymphocytes. *Environ. Mol. Mutagen.*, **26**, 292–304.
- Hilscher, W., Ohnesorge, F.K. & Müller, L. (1992) The effects of carbendazim on spermatogenesis. Unpublished report from Institut für Toxikologie, Heinrich-Heine-Universität, Düsseldorf, Germany. Previously submitted to WHO by Hoechst AG, Frankfurt, Germany.
- Hofmann, H.T. & Peh, J. (1973) Report on the testing of MCB (methyl-2-benzimidazole carbamate) for mutagenicity following intraperitoneal injection to the male mouse, Unpublished report Doc. No. A01398 from BASF Medizinisch-Biologische Forschungslaboratorien Gewerbehygiene und Toxikologie. Submitted to WHO by Bayer CropScience, Monheim, Germany.
- Hofmann, H.T. & Peh, J. (1987a) Report on the study to determine the prenatal toxicity of methyl benzimidazole-2-carbamate (MBC) in rats. Unpublished report No. 87/091, Doc. No. A52506 from BASF AG, Ludwigshafen, Germany. Previously submitted to WHO from BASF AG, Ludwigshafen, Germany.
- Hofmann, H.T. & Peh, J. (1987b) Report on the study to determine the prenatal toxicity of methyl benzimidazole-2-carbamate (MBC) in rats. Unpublished report No. 87/092, Doc. No. A52505 from BASF AG, Ludwigshafen, Germany. Previously submitted to WHO from BASF AG, Ludwigshafen, Germany.
- Hurt, M.E. (1993) Review of final report titled “The effects of carbendazim on spermatogenesis”. Unpublished report HLR 584-93 from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
- Jeffay, S.C., Libbus, B.L., Barbee, R.R. & Perreault, S.D. (1996) Acute exposure of female hamsters to carbendazim (MBC) during meiosis results in aneuploid oocytes with subsequent arrest of embryonic cleavage and implantation. *Reprod. Toxicol.*, **10**, 183–189.
- Marshall, R. (1996) Carbendazim: Induction of aneuploidy in cultured human peripheral blood lymphocytes. HLO 506-96 from Corning Hazleton, Harrogate, England. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
- Matsuo, F., Nakai, M. & Nasu, T. (1999) The fungicide carbendazim induces meiotic micronuclei in the spermatids of the rat testis. *J. Vet. Med. Sci.*, **61**, 573–576.
- Mayer & Kramer (1980) Testing of HOE 17411 – active ingredient (code HOE 17411 OF AT 204) for mutagenicity in the micronucleus test following oral administration to NMRI mice. Unpublished report

- No. 453/80, Doc. No. A29765 from Hoechst Pharma Forschung Toxikologie, Frankfurt, Germany. Submitted to WHO by Bayer CropScience, Monheim, Germany.
- Mayer, Weigand & Kramer (1980) Testing of HOE 17411 – active ingredient (Code HOE 17411 OF AT 207) for mutagenicity in the micronucleus test following oral administration to NMRI mice. Unpublished report No. 542/80, Doc. No. A28150 from Hoechst Pharma Forschung Toxikologie, Frankfurt, Germany. Submitted to WHO by Bayer CropScience, Monheim, Germany.
- Müller (1990) HOE 017411 – substance, technical and a mixture of HOE 017411 + HOE 093049 – substance, technical. Micronucleus test in male and female NMRI mice after oral administration. Unpublished report No. 89.0414, Doc. No. A42889 from Hoechst Pharma Research Toxicology and Pathology, Frankfurt, Germany. Submitted to WHO by Bayer CropScience, Monheim, Germany.
- Nakai, M., Hess, R.A., Moore, B.J., Guttroff, R.F., Strader, L.F. & Linder, R.E. (1992) Acute and long-term effects of a single dose of the fungicide carbendazim (methyl 2-benzimidazole carbamate) on the male reproductive system in the rat. *J. Androl.*, **13**, 507–518.
- Sarrif, A.M., Bentley, K.S., Fu, L.J., O’Neil, R.M., Reynolds, V.L. & Stahl, R.G. (1994) Evaluation of benomyl and carbendazim in the *in vivo* aneuploidy/micronucleus assay in DBF1 mouse bone marrow. *Mutat. Res.*, **310**, 143–149.
- Scholz & Schultes (1973) Report on a subchronic feeding experiment (93 days) with technical active substance HOE 17411 OF. Unpublished Doc. No. A00409 from Hoechst Pharma Fo. To., FRG. Submitted to WHO by Bayer CropScience, Monheim, Germany.
- Scholz & Weigand (1972) W17411 = 2-carbomethoxyaminobenzimidazol. Toxikologische Prüfung. Range-finding test (30 Tage) an Ratten. Unpublished Doc. No. A00011 from Hoechst Pharma Forschung Toxikologie. Submitted to WHO by Bayer CropScience, Monheim, Germany.
- Til, H.P., Leegwater, D.C. & Feron, V.J. (1971) Tentative (28-day) feeding study with W17411 in beagle dogs. Unpublished report No. R3659, Doc. No. A00015, from Central Institute for Nutrition and Food Research (TNO), the Hague, Netherlands. Submitted to WHO by Bayer CropScience, Monheim, Germany.
- Vanhauwaert, A., Vanparys, P. & Kirsch-Volders, M. (2001) The *in vivo* gut micronucleus test detects clastogens and aneugens given by gavage. *Mutagenesis*, **16**, 39–50.