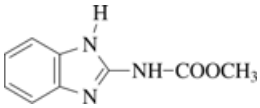
Carbendazim

Supplement 2011	
MAK value (2010)	**10 mg/m³ I**
Peak limitation (2010)	**Category II, excursion factor 4**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2010)	**Pregnancy Risk Group B**
Germ cell mutagenicity (2010)	**Category 5**
BAT value	**–**
Synonyms	1H-benzimidazol-2-ylcarbamic acid methyl ester carbendazole 2-(methoxycarbonylamino)benzimidazole
Chemical name (CAS)	methyl-1H-benzimidazole-2-ylcarbamate
CAS number	10605-21-7
Structural formula	
Molecular formula	$C_9H_9N_3O_2$
Molecular weight	191.2
Melting point	302–307 °C (decomposes) (EU 2007)
Boiling point	no data
Vapour pressure at 20°C	9×10^{-7} hPa (EU 2007)
log K_{OW}	1.5 at pH 5–7 (EU 2007)
Solubility	5–7mg/l water (pH 7); 28–36mg/l (pH 4) (EU 2007)
Purity	> 98%

The MAK-Collection Part I, MAK Value Documentations 2014
DFG, Deutsche Forschungsgemeinschaft

Impurities	2,3-diaminophenazine (DAP) (3 mg/kg), 2-amino-3-hydroxyphenazine (HAP) (0.5 mg/kg) in technical-grade products used as agricultural pesticides, up to 1994 the occurrence during synthesis of aminophenazine, which causes point mutations (documentation "Carbendazim" 2006)
Use	fungicide for plant protection and in silicone filling material, and facade and wood protectors

Documentation is available for the germ cell mutagenicity of carbendazim (documentation "Carbendazim" 2006). In this supplement, the other toxicological end points are considered. Unpublished studies are quoted from the reviews of the WHO (WHO 1993), the Joint Meeting on Pesticide Residues (JMPR 1995 a, 2005) and the EU (EU 2007). Carbendazim is a chemical with a high production volume whose main use is that of a fungicide. The fungicide benomyl is the metabolic precursor of carbendazim; for this reason the sections "Effects in humans" and "Carcinogenicity" include for assessment also studies with benomyl.

1 Toxic Effects and Mode of Action

The target organs of carbendazim are the liver, male germ cells, blood and the thyroid gland. In a 2-year feeding study, first effects on the body weights of dogs were found after doses of about 8 mg/kg body weight and day and above. The substance is aneugenic and at 100 mg/kg body weight and day, like other benzimidazoles, causes aneuploidy and polyploidy in female and male germ cells of the mouse in vivo and also in somatic cells. In rats, carbendazim is teratogenic after doses of 19.1 mg/kg body weight and above and has androgenic effects. Liver tumours occurred in two of three strains of mouse but not, however, in two strains of rat. The substance is not irritating to either the skin or eyes and is non-sensitizing. Unlike other carbamates, carbendazim is not a cholinesterase inhibitor.

2 Mechanism of Action

The aneugenic effects of carbendazim are caused by the binding of the substance to tubulin proteins, which disrupts microtubule assembly and the formation of spindles at cell division, thus resulting in the malsegregation of chromosomes. For this mechanism of action there is a dose below which there are no effects (JMPR 2005),

and a NEL (no effect level) of 50 mg/kg body weight was calculated for mice ("Carbendazim" 2006). The prenatal toxicity could likewise be attributed to disturbances in cell division, in addition to androgenic effects in the female offspring of rats (Lu et al. 2004).

The spermatotoxicity of carbendazim is linked with the effects on the androgen receptor (Lu et al. 2004), changed hormone concentrations (Rehnberg et al. 1989) and the disturbances in the formation of the microtubules (Nakai et al. 2002) and cytoskeleton (Yu et al. 2009) in the Sertoli cells of the testes.

The cause of the liver tumours in strains of mouse with a high incidence of spontaneous liver tumours is thought to be strain-specific toxic effects of carbendazim on the liver which lead to the promotion of tumours (JMPR 1995 a). In addition, carbendazim induces metabolizing liver enzymes (JMPR 1995 a; WHO 1993). The capacity of mice to detoxify carbendazim is lower than that of rats (JMPR 1995 a); this results in a higher level of exposure to carbendazim metabolites in the liver of mice and possibly also contributes to the higher sensitivity of mice compared with that of rats. No cholinesterase inhibition, such as can be assumed for a carbamate, has been detected (JMPR 1995 a; WHO 1993).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Male and female rats were given gavage doses of radioactively labelled carbendazim of 50 or 1000 mg/kg body weight. After 50 mg/kg body weight, 62% to 66% of the dose was eliminated within 72 hours with the urine in males and 54% to 62% in females; in the high dose group 41% of the dose was eliminated in both sexes. The rest was found in the faeces (JMPR 1995 a). On the basis of the radioactivity eliminated in the urine and the carbendazim metabolites in the faeces, oral absorption was determined to be at least 80% (see JMPR 1995 a in Section 3.2).

The substance and its metabolites are distributed throughout the whole body, with the highest concentrations in the liver and kidneys (EU 2007).

After single carbendazim doses of 3 or 300 mg/kg body weight, 12% to 18% of the dose was found in the liver of rats and 26% to 29% in the liver of mice after six hours. Examination of the animals' livers revealed that after carbendazim doses of 3 mg/kg body weight and day for 29 days, 2% of the dose was detected in the rat liver and below 2% in the mouse liver, while after doses of 300 mg/kg body weight and day, 4% of the dose was found in the liver of rats and 28% in that of mice (JMPR 1995 a). Rats are therefore able to eliminate repeated high doses of carbendazim, whereas mice are not. After repeated administration, the detoxification and elimination of carbendazim and its metabolites took place more rapidly in rats than in mice, which was reflected also in the increased glutathione level in the liver and the greater activity of phase II enzymes in rats (JMPR 1995 a).

After single intravenous doses of carbendazim of 12 mg/kg body weight, the half-lifes in the blood and in liver of rats were 0.1 and 0.16 hours, respectively, for the α phase and 2.15 and 6.15 hours for the β phase. The half-life for elimination with the urine was 12 hours. After oral administration of the same dose, 85% was bioavailable. Metabolites in the faeces were, however, not investigated (Krechniak and Klosowska 1986). In one rat, 83.5% of an oral dose was eliminated with the urine and 9.6% with the faeces after 24 hours (Gardiner et al. 1974).

Less than 1% of the oral dose was found in the rest of the body in rats after 72 hours (JMPR 1995 a).

After a single dose of 0.6 mg carbendazim was applied to the skin of rats, 0.2% of the dose was eliminated with the faeces and urine within 24 hours. When 60 mg carbendazim was applied, the absorbed amount was 0.03% (JMPR 1995 a). In a volunteer, only about 15% of a single oral dose of 2 mg carbendazim was eliminated with the urine in the form of 5-hydroxymethyl benzimidazole carbamate (5-HBC) after 24 hours. As about 15% was eliminated with the urine in the form of 5-HBC also in volunteers given intravenous doses, the authors concluded that carbendazim is 100% bioavailable after oral administration (Meuling et al. 1993). Most of the carbendazim and its metabolites are presumably eliminated with the faeces; this was, however, not investigated. The formation of other metabolites was also not investigated; the toxicokinetic data for carbendazim in humans is therefore inadequate.

Open or occlusive application of 0.3 ml of a suspension of 50 mg carbendazim per ml of a 50% aqueous ethanol solution to the skin of volunteers over an area of 100 cm^2 showed that about 0.4% of the dose was eliminated with the urine in the form of 5-HBC. This corresponds to the absorption of 3% carbendazim (Meuling et al. 1993). From this, an absorption rate for carbendazim of 1.1 $\mu g/cm^2$ and hour can be calculated. With a skin area of 2000 cm^2 and exposure for one hour, 2.2 mg carbendazim would therefore be absorbed. Calculations using the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995) show that, with a saturated aqueous solution, considerably lower quantities of 0.02 to 0.2 mg would be absorbed dermally.

3.2 Metabolism

In an unpublished study, the main metabolite found in male rats after single or repeated carbendazim doses of 50 mg/kg body weight or single doses of 1000 mg/kg body weight was 2-[[(methoxycarbonyl)amino]-1H-benzimidazole-5-yl]hydrogen sulfate (5-HBC-S) in amounts of 21% to 43%. In female rats, this metabolite made up 5.5% to 10%; the main metabolite is methyl(6-hydroxy-5-oxo-5H-benzimidazole-2-yl)carbamate-*N*-oxide (5,6-HOBC-*N*-oxide) (10% to 19%). In addition, various ring hydroxylated metabolites or their conjugates were found. After doses of 1000 mg/kg body weight, 10% to 15% was found in the faeces in the form of unchanged carbendazim. It was not specified whether unchanged carbendazim was

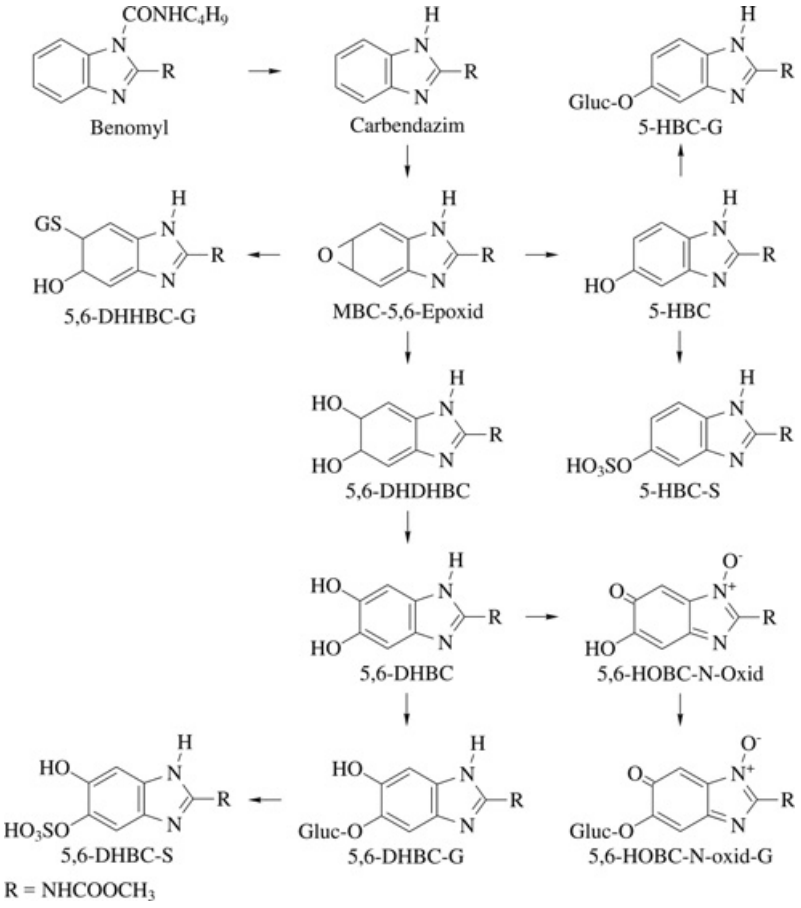


Figure 1 Metabolism of carbendazim and benomyl (according to JMPR 1995 a)

5,6-DHBC: methyl(5,6-dihydroxy-1*H*-benzimidazole-2-yl)carbamate

5,6-DHBC-G: 6-hydroxy-2-[[(methoxycarbonyl)amino]-1*H*-benzimidazole-5-yl]glucuronide

5,6-DHBC-S: 6-hydroxy-2-[[(methoxycarbonyl)amino]-1*H*-benzimidazole-5-yl]hydrogen sulfate

5,6-DHDHBC: 5,6-dihydro-5,6-dihydroxy-2-(methoxycarbonylamino)-1*H*-benzimidazole

5,6-DHHBC-G: *S*-[5,6-dihydro-5-hydroxy-2-(methoxycarbonylamino)-1*H*-benzimidazole-6-yl] glutathione

5,6-HOBC-N-oxide: methyl(6-hydroxy-5-oxo-5*H*-benzimidazole-2-yl)carbamate-*N*-oxide

5,6-HOBC-N-oxide-G: 2-[[(methoxycarbonyl)amino]-6-oxo-6*H*-benzimidazole-5-yl]glucuronide

5-HBC: methyl(5-hydroxy-1*H*-benzimidazole-2-yl)carbamate

5-HBC-S: 2-[[(methoxycarbonyl)amino]-1*H*-benzimidazole-5-yl]hydrogen sulfate

5-HBC-G: 2-[[(methoxycarbonyl)amino]-1*H*-benzimidazole-5-yl]glucuronide

MBC-5,6-epoxide: carbendazim-5,6-epoxide

found in the faeces after 50 mg/kg body (JMPR 1995 a). Therefore, at the low dose, absorption could also have been higher. Figure 1 shows the metabolism of carbendazim and benomyl.

Enzyme induction

In Wistar rats and Swiss mice, carbendazim concentrations of 2000 to 10^000 mg/kg diet (doses of about 200 to 1000 mg/kg body weight and day) caused increased liver weights and induced the activity of 7-ethoxycumarin-*O*-deethylase (CYP1A1/2, CYP2E1), biphenyl-4-hydroxylase (CYP2B1), aniline hydroxylase (CYP2E1), 4-methoxybiphenyl-*N*-demethylase, cytochrome c reductase and glucuronyl transferases I and II. In addition, the level of glutathione in the liver was increased. The increase in phase II enzymes was less pronounced in mice than in rats. A similar investigation revealed the induction of epoxide hydrolase, 7,8-styrene epoxide hydrolase and glutathione *S*-transferase in rats and mice. The total CYP level was not increased (JMPR 1995 a).

4 Effects in Humans

In 50 male workers exposed to benomyl or carbendazim, the leukocyte count, erythrocyte count, haemoglobin and haematocrit were unchanged compared with the values in 48 control persons (no other details) (JMPR 1995 a).

In the spouses of 298 male workers who produced benomyl, the number of births was not reduced compared with that in four control populations. Spermatogenesis was not investigated (JMPR 1995 a).

In 47 female fruit farm workers with dermatitis employed in sorting apples, patch testing produced one positive reaction to 5% carbendazim in ethanol. Of the women from the two control groups, consisting of 30 women from the same region possibly occasionally exposed while harvesting fruit and 60 women from another region undergoing minor surgical treatment as outpatients at a dermatological clinic, one from each group produced a positive reaction (no other details; Recchia et al. 1993). Of 122 fruit farmers in Taiwan, 46 reported “considerable” skin contact with carbendazim. They were, however, not tested with carbendazim (Guo et al. 1996).

5 Animal Experiments and in vitro studies

5.1 Acute toxicity

5.1.1 Inhalation

The LC_{50} for rats is above 5900 mg/m^3 (exposure duration not specified) (JMPR 1995 a; WHO 1993).

5.1.2 Ingestion

The oral LD_{50} for rats is above 10^000 mg/kg body weight (JMPR 1995 a).

5.1.3 Absorption through the skin

The dermal LD_{50} is above 2000 mg/kg body weight in rats (JMPR 1995 a; WHO 1993) and above 10 000 mg/kg body weight in rabbits (WHO 1993).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

There are no studies available.

5.2.2 Ingestion

Only studies relevant to the evaluation are listed below. Details of long-term studies in mice and rats can be found in Table 2 in Section 5.7.

Mouse

In a 2-year feeding study with groups of 80 male and 80 female CD-1 mice, the absolute and relative thymus weights were reduced in the females at the lowest carbendazim concentration of 500 mg/kg diet (doses of about 60 mg/kg body weight and day) and above. The absolute kidney and thymus weights were decreased in the males. At 1500 mg/kg diet (doses of about 180 mg/kg body weight and day) and above, centrilobular hypertrophy, necrosis and swelling of the liver, lymphoid depletion in the thymus and a yellow-brown pigment in the renal tubules were observed in the males. The relative liver weights of the female mice were increased. In the males, the highest concentration of 7500 mg/kg (reduced to 3750 mg/kg for the males after 66 weeks, corresponding to doses of about 400 mg/kg body weight and day) produced a decrease in food efficiencya reduced erythrocyte count, cystic kidney tubules, increased mortality and decreased relative kidney and thymus weights. In the females of this group, the absolute thymus weights were not decreased while the absolute liver weights were increased (JMPR 1995 a; WHO 1993). No systemic NOAEL (no observed adverse effect level) can be derived from this study, as increased incidences of liver tumours occurred in the female mice at 500 mg/kg diet (doses of about 60 mg/kg body weight and day) and above (see Section 5.7). Nor can a NOAEL be derived from an 80-week study with groups of 100 male and 100 female Swiss mice, as hyperplasia occurred at the low carbendazim concentration of 150 mg/kg diet (doses of about 19 mg/kg body weight and day)

and above, and tumours in the liver at 300 mg/kg diet (doses of about 37 mg/kg body weight and day) and above. At 5000 mg/kg diet (doses of about 600 mg/kg body weight and day), the relative liver weights were increased, although body weights were unchanged (JMPR 1995 a; WHO 1993). Groups of 100 to 120 male and female HOE:NMRKf mice were given a diet containing carbendazim in concentrations of 0, 50, 150, 300 or 1000 mg/kg (♂/♀: doses of 5.8/7.1, 17.1/21.2, 34.4/41.9, 548.4/682.3 mg/kg body weight and day) for 96 weeks. The highest concentration was increased to 2000 mg/kg diet after 4 weeks and to 5000 mg/kg after 8 weeks. 20 male and 20 female animals of the high concentration group were investigated after 18 months. Centrilobular hypertrophy, single cell necrosis, mitotic cells and pigmented Kupffer cells were found in the liver. All the organs of the remaining animals were subjected to histopathological examination after 22 months. In the mice of the high dose group investigated after 22 months, the liver findings were more pronounced than after 18 months, and the absolute and relative liver weights were increased. Mortality and body weight development were unchanged compared with that in the controls. The NOAEL was 300 mg/kg diet (34 mg/kg body weight and day), the LOAEL (lowest observed adverse effect level), which caused liver toxicity and increased liver weights, was 5000 mg/kg (550 mg/kg body weight and day) (JMPR 1995 a; WHO 1993).

Rat

Rats were given gavage doses of carbendazim of 0, 150, 300 or 600 mg/kg body weight and day for 15 weeks. Histological changes in the thyroid and parathyroid and the adrenal glands were observed after 150 mg/kg body weight and day and above. The triiodothyronine level was significantly increased at 300 mg/kg body weight and day. The levels of thyroxine, thyroid stimulating hormone (TSH), adrenocorticotropic hormone and growth hormone were unchanged (Barlas et al. 2002).

Groups of 36 male and 36 female CD rats were given test substance with the diet in concentrations of 0, 100, 500, 5000 or 2500 mg/kg (increased to 10^000 mg/kg after 20 weeks) (doses of about 0, 3, 15, 150, 300 mg/kg body weight and day) for 104 weeks. The test substance contained 50% to 70% carbendazim (no other details). Clinico-chemical, haematological and urine investigations were carried out. Gross pathological and microscopic examinations were carried out in 6 animals from each concentration group after one year. At the end of the study, all the organs of the high dose and control groups, and the liver in all the animals, were examined histologically. The relative liver weights were increased in the female rats after 5000 mg/kg diet and above, while body weight gains, and the erythrocyte count, haematocrit and haemoglobin values were decreased. At 10^000 mg/kg these effects were found also in the males, in addition to diffuse testicular atrophy and prostatitis (JMPR 1995 a; WHO 1993). A NOAEL of 500 mg/kg diet (doses of about 15 mg/kg body weight and day) and a LOAEL of 5000 mg/kg diet (doses of about 150 mg/kg body weight and day) can be derived for the effects on body weight gain and blood.

In another study, groups of 60 male and 60 female Wistar rats were given a diet containing carbendazim (purity 99%) in concentrations of 0, 150, 300 or 2000 mg/kg (increased to 10 000 mg/kg after two weeks) (doses of about 0, 7.5, 15, 500 mg/kg body weight and day) for two years. Clinico-chemical, haematological and urine investigations were carried out. All animals were subjected to gross pathological examination and 20 animals per sex were examined histologically in the control and high dose groups. Fifty percent of the high dose animals survived up to 92 weeks. In the females of the high dose group, the haematocrit, haemoglobin and serum protein levels, and body weight gains were decreased. The alanine aminotransferase (ALAT) activity, relative liver weights and the incidence of diffuse proliferation in parafollicular cells of the thyroid were increased. The only effect in the males of this dose group was a decrease in aspartate aminotransferase (ASAT) activity (JMPR 1995 a; WHO 1993). In this study the systemic NOAEL was 300 mg/kg diet (doses of about 15 mg/kg body weight and day) and the LOAEL 10^000 mg/kg diet (doses of about 500 mg/kg body weight and day), which caused effects on body weight gain, the liver, thyroid and blood.

Dog

Groups of 5 beagles per sex were given carbendazim with the diet in concentrations of 0, 100, 200 or 500 mg/kg (doses of about 0, 3, 6, 15 mg/kg body weight and day) for one year. In the high dose group, a statistically significant increase in cholesterol levels was observed in the males only after nine months and in the females only after one to two months. There were no effects on food consumption, body weights, clinical pathology and histologically investigated organs (no other details) (JMPR 1995 a; WHO 1993). As the effect on the cholesterol level was only transient, the NOAEL is 500 mg/kg diet (doses of about 15 mg/kg body weight and day).

In a 2-year study, groups of 4 male and 4 female animals were given carbendazim with the diet in concentrations of 0, 100, 500 or 2500 mg/kg (doses of about 0, 3, 15, 75 mg/kg body weight and day). One male and one female from both the control and the 500 mg/kg group were investigated after one year. Three male animals of the high dose group were sacrificed after 22 and 42 weeks because of poor nutrition. The dogs of the 500 mg/kg group had increased levels for cholesterol, blood urea nitrogen, total protein and ALAT. The histopathological findings were not clearly presented. While, according to WHO (1993), no histopathological effects were reported at 500 mg/kg diet, inflammatory and fibrotic changes in the liver were observed at 2500 mg/kg diet; however, the authors did not consider these to be clearly substance-related on account of "uncertainties in dosage, exposure time and the number of animals". JMPR (1995 a) attributes these effects to concentrations of 500 mg/kg diet and above. There were no effects on organ weights, haematology and urine values (JMPR 1995 a; WHO 1993). A NOAEL cannot be established as the original study is not available. In another 2-year study, groups of 4 male and 4 female beagles were given carbendazim in the diet in concentrations of 0, 150, 300 or 2000 mg/kg (increased to 5000 mg/kg after 33 weeks) (doses of about 4, 8, 150 mg/kg body weight and day). Food intake, body weights, urine,

blood, liver and kidney function and organs were investigated (gross pathological and microscopic examination). The body weights of the males were reduced after 300 mg/kg and above. In the high dose group, the body weights of the females were decreased. In both sexes the blood coagulation time was reduced. The alkaline phosphatase activity and the absolute and relative liver and thyroid weights were increased, as were the incidences of prostatitis and atrophy of the seminal tubules. No microscopically detectable effects were found in the liver, and neither ALAT nor ASAT were changed. The authors derived a NOAEL of 300 mg/kg diet (WHO 1993). JMPR (1995 a) considers the description of the pathological findings as inadequate for establishing a NOAEL. If the reduction in body weights at 300 mg/kg diet (8 mg/kg body weight and day) is regarded as the LOAEL, a NOAEL of 150 mg/kg diet (4 mg/kg body weight and day) can be derived.

Summary

In a long-term study with NMRKf mice, a NOAEL of 34 mg/kg body weight and day was obtained, while hyperplasia of the liver occurred after doses of as little as 19 mg/kg body weight and day in the more sensitive Swiss mice. The NOAEL for rats was 15 mg/kg body weight and day in two 2-year studies; increased relative liver weights and erythrocytopenia with reduced haemoglobin and haematocrit values occurred at 150 mg/kg body weight and day. In the dog, the NOAEL was about 4 mg/kg body weight and day, also after exposure for two years, while body weight gains were reduced at the LOAEL of 8 mg/kg body weight and day.

5.2.3 Absorption through the skin

In a 10-day study with rabbits, a 50% aqueous paste was applied for 6 hours a day. The systemic NOAEL was 2000 mg/kg body weight and day. Although focal necrosis of the epidermis occurred, there were no gross pathological or microscopically observable adverse effects (EU 2007; WHO 1993).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

In a patch test with rabbits over 4 hours, 5 g of a 50% product (wettable powder, probably containing tensides) was described as not irritating to the skin (EU 2007; JMPR 1995 a; WHO 1993).

A 75% carbendazim formulation (wettable powder, probably containing tensides) caused slight transient irritation after application to the rabbit skin (exposure duration not specified). A 55% suspension of a 75% carbendazim formulation in dimethyl phthalate caused slight irritation to the skin of guinea pigs. The suspension was not irritating in concentrations of 5.5% (JMPR 1995 a).

5.3.2 Eyes

Technical-grade carbendazim was not irritating to the eyes of rabbits (no other details) (JMPR 1995 a; WHO 1993). 75% (JMPR 1995 a) and 50% (JMPR 1995 a; WHO 1993) powdered carbendazim formulations (wettable powder, probably containing tensides) caused irritation to the eyes of rabbits; this was attributed to the inert ingredients in the formulation (JMPR 1995 a).

5.4 Allergenic effects

A modified maximization test in guinea pigs yielded no evidence that carbendazim has contact allergenic potential. Induction was carried out via intradermal injection of Freund's complete adjuvant, 5% carbendazim in olive oil and 5% carbendazim in olive oil with Freund's complete adjuvant. Epicutaneous provocation with 25% carbendazim in petrolatum after pretreatment with 10% sodium lauryl sulfate did not produce any reactions. Corresponding tests for cross-reactions with 2-mercaptobenzimidazole, 2-mercaptobenzimidazole analogues and ethylene thiourea also yielded negative results (no other details were obtainable from the Japanese text; Shimizu et al. 1994). An investigation of the sensitizing effects of technical-grade carbendazim and a 75% powdered formulation (no other details) in 10 male guinea pigs did not yield any evidence of contact sensitizing effects after either intradermal injection or repeated application to the shaved intact skin (no other details; WHO 1993). In a maximization test with intradermal and topical induction with 5% or 25% carbendazim in water, carbendazim (no other details) did not produce a reaction in any of the 10 female Hartley guinea pigs, 24 and 48 hours after epicutaneous provocation with concentrations of 1% or 5% carbendazim in water. Two and three of the animals pretreated during induction with carbendazim reacted after provocation to 0.5% and 2% benomyl, respectively (Matsushita et al. 1977). Negative results were obtained also in a Bühler test with a 50% carbendazim preparation in 80% ethanol in 10 male and 10 female Hartley guinea pigs (WHO 1993).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

Generation studies

In a one-generation study, groups of 8 to 12 male and female Long-Evans rats were given gavage doses of carbendazim of 0, 50, 100, 200 or 400 mg/kg body weight and day from weaning (day 21 after birth) through puberty, mating (male animals investigated on days 104 to 106) and gestation up to the end of lactation (investigation of the females 25 days after giving birth). In the parent generation, carbendazim did

not alter development during puberty, body weight gains, the weights of the liver, kidneys, adrenal glands, ovaries and pituitary gland, or mortality. The sperm count in the tail of the epididymis was reduced after 50 mg/kg body weight and day and above, and slight testicular atrophy occurred. At 100 mg/kg body weight and day and above the litter sizes were reduced and the number of resorptions increased. These effects were not statistically significant according to Gray et al. (1990). An increase in the number of abnormal sperms was observed. Externally visible malformations (hydrocephalus and tail malformations) occurred sporadically. At 200 mg/kg body weight and day and above, around 50% of the male rats were infertile. The sperm count, sperm motility, and testis and epididymis weights were reduced, and foetal mortality was increased. The levels of testosterone and testosterone stimulated by human chorionic gonadotropine and of androgen binding protein (ABP), luteinizing hormone (LH) and follicle stimulating hormone (FSH) were increased, especially in animals with a low sperm count (Gray et al. 1988, 1990).

A similar study in male and female Syrian hamsters with 0 and 400 mg/kg body weight and day yielded similar findings in the animals treated with carbendazim. They were, however, less pronounced than in rats (Gray et al. 1990). In a three-generation study with two litters per generation, groups of 3 male and 16 female Sprague Dawley rats (high dose group 20 female animals) were given carbendazim with the diet in concentrations of 0, 100, 500, 5000 or 10^000 mg/kg, corresponding to doses of about 5 to 500 mg/kg body weight and day. Carbendazim had no effects on fertility, gestation, viability and lactation. Histopathological examination of 4 offspring of 5 dams of the second litter of the F_2 generation revealed no adverse effects. At 250 mg/kg body weight and day and above, the average weight of the litters at the time of weaning was reduced (JMPR 1995 a; WHO 1993). The NOAEL for toxic effects on postnatal development in this study is 25 mg/kg body weight and day, the LOAEL 250 mg/kg body weight and day, and the NOAEL for fertility 500 mg/kg body weight and day.

In a three-generation study with two litters per generation, groups of 10 male and 20 female Wistar rats were given carbendazim with the diet in concentrations of 0, 150, 300 or 2000 mg/kg. All animals treated with carbendazim weighed more than the control animals by the end of the study. Carbendazim had no effects on fertility, postnatal mortality and body weight gains up to day 20 after birth (JMPR 1995 a). The NOAEL for toxic effects on postnatal development and fertility is 2000 mg/kg diet, corresponding to doses of about 120 mg/kg body weight and day.

The fact that (unlike in the gavage study) no influence on fertility was observed in the two generation studies in which carbendazim was administered with the diet–even at the highest doses of 500 mg/kg body weight and day (JMPR 1995 a; WHO 1993) and 120 mg/kg body weight and day (JMPR 1995 a)–is thought to be connected with the continuous absorption, as opposed to bolus administration.

Studies of male fertility

The following describes a selection of mechanistic studies of the effects of carbendazim on the endocrine system and fertility. Male Wistar rats were given carbendazim

with the diet in concentrations of 0, 10, 70 or 500 mg/kg for 182 days. No effects on fertility parameters, testis weights, the surface area of the seminiferous tubules and the interstitial tissue, epididymal structures or the activities of the enzymes thiamine pyrophosphatase, ALAT, alkaline phosphatase, Ca-ATPase, Mg-ATPase and various steroid dehydrogenases were observed up to the high dose. The NOAEL was 500 mg/kg diet, corresponding to carbendazim doses of 29 to 43 mg/kg body weight and day (JMPR 2005).

Carbendazim was administered by gavage to male Wistar rats in daily doses of 0, 20, 100 or 200 mg/kg body weight and day 80 days prior to mating. At 100 mg/kg body weight and day and above, the fertility index, testis weights, and sperm count and motility were reduced. The seminiferous tubules were atrophic, the number of germ cells was reduced. Carbendazim inhibited meiotic transformation and spermiogenesis. At 200 mg/kg body weight and day there was a statistically significant reduction in the LH level. FSH and testosterone were unaffected. The NOAEL for male fertility was 20 mg/kg body weight and day (Yu et al. 2009). Twenty-four male Sprague Dawley rats were given gavage doses of carbendazim of 400 mg/kg body weight and day for 10 days; 24 untreated animals were used as controls. Following the third dose, the animals were mated every week for 32 weeks. Fertility was reduced in the first week after the end of exposure: 10 of the treated animals were infertile during mating in the first week, 16 after the 5th week. Only 4 of these infertile animals recovered during the further course of the study. The infertile rats were found to have severe atrophy of the seminiferous tubules, in which only Sertoli cells were present in the seminiferous epithelium. In the animals that recovered, the atrophy was less pronounced (WHO 1993).

The decrease in testis weights, spermatozoa and sperm concentrations and the change in sperm morphology observed in Sprague Dawley rats after daily gavage doses of carbendazim of 675 mg/kg body weight and day for 28 days could be prevented by the simultaneous administration of the androgen receptor antagonist flutamide (Lu et al. 2004).

In male rats, daily gavage doses of carbendazim of 25, 50, 100, 200, 400 or 800 mg/kg body weight and day for 56 days increased the androgen receptor concentration in the testes and epididymis. Carbendazim displaced 5-alpha-dihydrotestosterone from the androgen receptor (Lu et al. 2004).

To investigate the influence of carbendazim on endocrine functions, Long-Evans rats were given gavage doses of carbendazim of 0 to 400 mg/kg body weight and day for 85 days. Doses of 50 to 100 mg/kg body weight and day had no influence on the concentrations of LH, FSH, TSH, prolactin, ABP and testosterone. After doses of 200 mg/kg body weight and day and above, the concentrations of testosterone and ABP were increased in the fluid of the seminiferous tubules, but not in the serum. At 400 mg/kg body weight and day, the concentrations of testosterone and ABP were increased also in the interstitial fluid, and the concentration of ABP was increased in the serum. These findings can be attributed to the increased release of testosterone by the Leydig cells or the reduced release of testosterone from the testes into the blood circulation, as well as a change in the

relative secretion of ABP into the interstitial fluid and the seminiferous tubules (Rehnberg et al. 1989).

In another study with the same doses as in the study described above, a dose dependent increase in the concentrations of FSH and LH in the serum and a decrease in the concentration of gonadotropine-releasing hormone in the hypothalamus was observed in Long-Evans rats. The direct toxicity of carbendazim in the testes is accompanied by additional compensatory changes in the hypothalamus and pituitary gland (Goldman et al. 1989).

Single doses of carbendazim of 400 mg/kg body weight in male Sprague Dawley rats produced stage-specific sensitivity of the microtubules in the Sertoli cells of the testes (Nakai et al. 2002).

In a study in which male Wistar rats were given single doses of carbendazim of 400 mg/kg body weight, pachytene spermatocytes were found to be absent in the tubules at stages III–IV. In addition, a selective loss in step 14 spermatids, asynchrony of the stages in the spermatogenic cycle and the development of Sertoli cell fibrosis in the seminiferous tubules occurred. The effects of carbendazim thus depended on the stage of the spermatogenic cycle during exposure (Kadalmani et al. 2002).

Studies of female fertility

Female Holtzman rats were given gavage doses of carbendazim of 0, 25, 50, 100, 200, 400 or 1000 mg/kg body weight and day during the first 8 days of gestation. On day 9, reductions in maternal body weight gains, the weight of the implantation sites (number of implantation sites not specified) and in serum LH, and increased serum oestradiol levels were found in the high dose group. The levels of serum progesterone and serum prolactin were unchanged, as was the number of resorptions. Pseudopregnant rats were given carbendazim doses of 0 or 400 mg/kg body weight and day. Uterine decidual growth was reduced in the treated animals (Cummings et al. 1990).

Female Syrian hamsters were given single gavage doses of carbendazim of 0, 250, 500, 750 or 1000 mg/kg body weight during meiosis I. At 250 mg/kg body weight and above, the number of preimplantation losses was increased and the litter sizes reduced, at 500 mg/kg body weight and above an increase in early and mid-term resorptions occurred. At 750 mg/kg body weight and above, the number of pregnant animals and the number of live pups were significantly reduced. Fertility was unaffected by the administration of 1000 mg/kg body weight during meiosis II, but the number of live pups was reduced. This study shows that carbendazim can impair microtubule-dependent events such as meiosis, which in this case results in early losses during pregnancy (Perreault et al. 1992).

Summary

Carbendazim caused histological changes in the testes of male Long-Evans rats at 50 mg/kg body weight and day and above (Gray et al. 1990) and impairment of the

fertility of male Wistar rats at 100 mg/kg body weight and day and above. A NOAEL for male fertility of 20 mg/kg body weight and day was obtained (Yu et al. 2009). Female fertility was not affected in Holtzman rats until doses of 1000 mg/kg body weight and day (Cummings et al. 1990). In female hamsters, however, fertility was impaired after doses of as little as 250 mg/kg body weight and day after specific treatment during meiosis I (Perreault et al. 1992).

5.5.2 Developmental toxicity

In male and female Sprague Dawley rats given gavage doses of carbendazim of 200 mg/kg body weight and day Sprague Dawley for 28 days followed by a subsequent treatment-free mating period, no histological changes were found in the testes of the male offspring. However, androgenic effects such as incomplete development of the uterine horn, an enlarged urethra, the absence of the vagina and the induction of seminal vesicles were observed in the female offspring (Lu et al. 2004). Thestudies of the toxic effects of carbendazim on prenatal development are shown in Table 1.

Table 1 Studies of the toxic effects of carbendazim on prenatal development

Species, strain, number of animals per group	Exposure	Findings	References
rat, Imp: Lodz, 20–23 ♀	**GD 6–15** 0, 8, 35, 160 mg/kg body weight and day, gavage, examination on GD 20	**8 mg/kg body weight**: dams, foetuses: NOAEL **35 mg/kg body weight and above**: dams: toxicity; foetuses: lethal to embryos, congenital defects (encephalocele, umbilical hernia, missing or shorter tails, malformations of brain, kidneys, ribs, costal arch, vertebrae), delayed foetal development	Sitarek 2001
rat, Sprague Dawley, 25 ♀	**GD 7–16** 0, 5, 10, 20, 90 mg/kg body weight and day, gavage, examination on GD 22	**10 mg/kg body weight**: foetuses: NOAEL **20 mg/kg body weight and above**: dams: NOAEL; foetuses: foetal weights decreased **90 mg/kg body weight**: dams: body weight gains decreased, absolute and relative liver weights increased; *foetuses*: early resorptions increased, litter sizes decreased, total resorptions (3 litters), malformations (hydrocephalus, microphthalmia, anophthalmia, scapular and axial skeletal malformations: vertebral, rib, and sternebral fusions, exencephaly, hemivertebrae, rib hyperplasia)	JMPR 1995 a, 2005; WHO 1993

Table 1 (Continued)

Species, strain, number of animals per group	Exposure	Findings	References
rat, Sprague Dawley, 15–26 ♀	**GD 6–15** 0, 10, 30, 60, 100, 300, 1000, 3000 mg/kg body weight and day, gavage, examination on GD 20	**10 mg/kg body weight and above**: foetuses: foetal weights decreased compared with weights of the vehicle controls, but not of the untreated controls **30 mg/kg body weight and above**: dams: NOAEL; *foetuses*: number of malformations increased **60 mg/kg body weight and above**: dams: dose-dependent decrease in body weight gains; foetuses: embryo mortality increased	JMPR 2005
rat, Sprague Dawley, 20–30 ♀	**GD 6–15** 0, 10, 30 mg/kg body weight and day, gavage, examination on GD 20	**10 mg/kg body weight and above**: foetuses: NOAEL, number of variations increased (dilation of brain ventricles) **30 mg/kg body weight**: dams: NOAEL; oetuses: foetal weights decreased, number of runts increased, malformations increased (ribs, spine, head), variations increased (sternebral aplasia, displacement of sternebrae)	JMPR 2005
rat, Sprague Dawley, 11 ♀	**GD 8–15** 0, 19.1 mg/kg body weight and day, gavage, examination on GD 21	**19.1 mg/kg body weight**: foetuses: weights decreased, mortality increased, skeletal and external malformations	Delatour and Besse 1990
rat, Wistar, 8–10 ♀	**GD 6–15** 0, 20, 40, 80 mg/kg body weight and day, gavage, examination on GD 21 or PND 21	**20 mg/kg body weight and above**: foetuses GD 21: number of resorptions increased; offspring: litter sizes slightly decreased **up to 80 mg/kg body weight**: foetuses GD 21: no malformations	Janardhan et al. 1984
rat, Holtzman, 9–11 ♀	**GD 1–8** 0, 100, 200, 400, 600 mg/kg body weight and day, gavage, examination on GD 11 or 20	**100 mg/kg body weight and above**: foetuses GD 20: number of resorptions increased, litter size decreased, mortality increased, delayed ossification **200 mg/kg body weight and above**: foetuses GD 11: body and litter sizes decreased, malformations (open neural tube, limbs)	Cummings et al. 1992

Table 1 (Continued)

Species, strain, number of animals per group	Exposure	Findings	References
rat, Sprague Dawley, 27–28 ♀	**GD 6–15** 0, 8.9, 45.9, 218.4, 431.6, 625.5, 746.9 mg/kg body weight and day, with the diet, examination on GD 20	**746.9 mg/kg body weight**: dams: food intake decreased; foetuses: no increase in the number of malformations	JMPR 1995 a, 2005; WHO 1993
rabbit, New Zealand White, 20 ♀	**GD 7–19** 0, 10, 20, 125 mg/kg body weight and day, gavage, examination GD not specified	**10 mg/kg body weigh**t: dams, foetuses: NOAEL **20 mg/kg body weight and above**: dams: implantations decreased (as the substance was not administered until after implantation had started it is questionable whether the effect is substance-related); foetuses: litter sizes decreased **125 mg/kg body weight**: dams. body weight gains decreased, number of resorptions increased; foetuses: resorptions increased, foetal weights decreased, malformations (cervical vertebrae, ribs, thoracic vertebrae)	JMPR 1995 a, 2005; WHO 1993
rabbit, "albino", no other details	GD 6–18 0, 40, 80, 160 mg/kg body weight and day, gavage, examination on "day" 31, natural birth	40 mg/kg body weight: offspring: number of resorptions increased	Janardhan et al. 1984

GD: gestation day, PND: postnatal day

In several studies of prenatal toxicity with gavage administration of carbendazim, Sprague Dawley rats were found to have malformations of the brain, kidneys, ribs, costal arch and vertebrae after doses of 19.1 mg/kg body weight and above (Delatour and Besse 1990) and 30 mg/kg body weight and above (JMPR 2005). These malformations were not found in a feeding study in Sprague Dawley rats with doses up to 746.9 mg/kg body weight and day (JMPR 1995 a, 2005; WHO 1993). This could indicate that it is not the dose, but the concentration of carbendazim that is decisive for the effects, which therefore become manifest only after bolus administration. At the same time, there appear to be also strain-related differences in sensitivity, as no malformations were found in Wistar rats after gavage administration

up to the highest dose tested of 80 mg/kg body weight and day (Janardhan et al. 1984). Prenatal toxicity (increased number of resorptions or reduced litter sizes) and foetotoxicity (reduced foetal weights) were however observed in Sprague Dawley rats after doses of 19.1 mg/kg body weight and day and above and also in Wistar rats and in rabbits after 20 mg/kg body weight and day and above. The NOAEL for developmental toxicity after gavage administration is 8 mg/kg body weight and day for rats (Sitarek 2001) and 10 mg/kg body weight and day for rabbits (JMPR 1995 a, 2005; WHO 1993). In another study, no foetotoxic or teratogenic effects were observed in the offspring of Wistar-SPF rats after carbendazim doses of 0, 600, 2000 or 6000 mg/kg diet from gestation days 6 to 15. However, the data were not related to the individual litters but given only as the percentage. The results of the study are therefore not included in the evaluation (WHO 1993). Also a study in rabbits with the same carbendazim concentrations is not included, as at 2000 mg/kg diet only three of 25 animals were pregnant and therefore too few litters were available for evaluation (JMPR 1995 a; WHO 1993).

5.6 Genotoxicity

In numerous studies carbendazim was not directly reactive with DNA. In 2005 carbendazim was classified in Category 3A for Germ Cell Mutagens (documentation "Carbendazim" 2006), as carbendazim causes diploidization and aneuploidy in female and male germ cells of rats and mice.

In the documentation from 2006, 0.5 and 8 µM were given as the NEL in vitro for aneuploidy and polyploidy, respectively. A NEL for aneuploidy in the mouse in vivo was calculated to be 50 mg/kg body weight and day, the corresponding LEL (lowest effect level) was 100 mg/kg body weight (documentation "Carbendazim" 2006).

5.7 Carcinogenicity

The studies of the carcinogenicity of carbendazim are shown in Table 2.

Mouse

Carbendazim (99%) was administered to groups of 80 male and 80 female CD-1 mice in concentrations of 0, 500, 1500 or 7500 mg/kg diet (corresponding to doses of about 60 to 900 mg/kg body weight and day) for two years. After 66 weeks the high concentration was reduced to 3750 mg/kg diet for the male animals on account of increased mortality (48/80, controls: 18/80). The male animals of this group were killed after only 73 weeks. Also in the 1500 mg/kg group, mortality and liver toxicity occurred in male mice (see Section 5.2.2). In the female animals of this group the relative liver weights were increased. At 60 mg/kg body weight and day

and above the incidence of hepatocellular tumours was significantly increased in the female animals, and at 180 mg/kg body weight and day in the male animals. Only the combined incidences were given (in tabular form) for the carcinomas, adenomas and hepatoblastomas. According to the authors, the incidence of adenomas was not increased. The increase in the tumour frequency was based on an increased incidence of carcinomas (no other details; JMPR 1995 a; WHO 1993).

Carbendazim was administered to groups of 100 male and 100 female Swiss mice in concentrations of 0, 150, 300 or 1000 mg/kg diet (about 0, 19, 37, 600 mg/kg body weight and day) for two years. The high concentration was increased to 5000 mg/kg diet after 8 weeks. After 37 mg/kg body weight and day and above, the incidence of liver tumours (adenomas and carcinomas combined) in both sexes was increased. The female animals had only liver adenomas and no carcinomas (JMPR 1995 a; WHO 1993). No statistical evaluation of the data was performed. Therefore, on the basis of the numerical increase in liver tumours at 37 mg/kg body weight and day, a NOAEL for tumours of 19 mg/kg body weight and day was derived. At this dose, however, hyperplasia in the liver was observed.

Groups of 100 to 120 male and female HOE:NMRKf mice were given carbendazim in concentrations of 0, 50, 150, 300 or 1000 mg/kg diet (♂/♀: 0/0; 5.8/7.1; 17.1/21.1; 34.4/41.9; 548.4/682.3 mg/kg body weight and day) for 96 weeks. The high concentration was increased to 2000 mg/kg in the 4th week and to 5000 mg/kg in the 8th week. 20 male and 20 female animals from the high concentration group and from the control group were examined after 18 months. Despite toxic effects on the liver in the animals of the high dose group (see Section 5.2.2), the incidence of liver tumours was not increased (Table 2). Also the incidence of lung tumours was not increased (JMPR 1995 a; WHO 1993). The NOAEL for tumours was about 600 mg/kg body weight and day.

Rat

In a 2-year study with groups of 30 male and 30 female ChR-CD rats, there was no increase in tumour incidences up to the highest concentration tested of 10^000 mg/kg diet (doses of about 300 mg/kg body weight and day) (JMPR 1995 a; WHO 1993). In a second long-term study with 60 male and 60 female Wistar rats (only 20 animals per sex were histopathologically investigated), there were likewise no increased tumour incidences up to the highest concentration of 10^000 mg/kg diet (doses of about 500 mg/kg body weight and day) (JMPR 1995 a; WHO 1993).

Summary

Carbendazim caused liver tumours in CD-1 and Swiss mice in concentrations of 300 and 500 mg/kg diet and above (doses of about 35 or 60 mg/kg body weight and day and above), but not, however, in HOE:NMRKf mice in doses up to 600 mg/kg body weight and day. The tumourigenic effects of carbendazim on the liver appear to be strain-specific and dependent on the frequency of spontaneous tumours, as the two sensitive strains have a higher frequency of spontaneous liver tumours than

Table 2 Studies of the carcinogenicity of carbendazim

Author	JMPR 1995 a; WHO 1993				
Substance	carbendazim, 99%				
Species	mouse, CD-1, groups of 80 ♂, 80 ♀				
Administration route	with the diet				
Concentration	0, 500, 1500, 7500–3750 mg/kg diet (doses of about 0, 60, 180, 400 mg/kg body weight and day, estimated using data from the study with HOE:NMRKf mice; see below)				
Duration	2 years				
Toxicity	180 mg/kg body weight and day and above: ♂: liver toxicity, ♀: relative liver weights increased				
		Dose (mg/kg body weight and day)			
		0	60	180	400
survivors after 2 years	♂	18/80 (23%)	14/80 (18%)	9/80 (11%)	not specified[a]
	♀	22/80 (28%)	15/80 (19%)	13/80 (16%)	20/80 (25%)
Tumours					
Liver					
total adenomas, carcinomas, blastomas	♂	13/80 (16%)	20/80 (25%)	23/80 (29%)*	not specified[a]
	♀	1/79 (1%)	9/78 (12%)*	21/80 (26%)*	15/78 (19%)*

* $p < 0.05$;
[a] all animals killed after 73 weeks because of high mortality

Author	JMPR 1995 a; WHO 1993				
Substance	carbendazim, purity not specified				
Species	mouse, Swiss, groups of 100 ♂, 100 ♀				
Administration route	with the diet				
Concentration	0, 150, 300, 1000–5000 mg/kg diet (doses of about 0, 19, 37, 600 mg/kg body weight and day, estimated using data from the study with HOE:NMRKf mice; see below)				
Duration	80 weeks				
Toxicity	19 mg/kg body weight and day and above: ♂, ♀: hyperplasia in the liver				
		Dose (mg/kg body weight and day)			
		0	19	37	600
survivors after 80 weeks	♂	70%	70%	70%	70%
	♀	80%	80%	80%	80%

Table 2 (Continued)

		Dose (mg/kg body weight and day)			
		0	19	37	600
Tumours and preneoplastic lesions					
Liver					
nodular hyperplasia	♂	0/100	8/94 (9%)	11/98 (11%)	25/100 (25%)
	♀	0/94	5/99 (5%)	3/98 (3%)	11/95 (12%)
adenomas	♂	9/100 (9%)	5/94 (6%)	13/98 (13%)	14/100 (14%)
	♀	1/94 (1%)	1/99 (1%)	3/98 (3%)	8/95 (9%)
carcinomas	♂	1/100 (1%)	3/94 (3%)	4/98 (4%)	9/100 (9%)
	♀	1/94 (1%)	0/99	0/98	0/95

No statistical evaluation given

Author	JMPR 1995 a; WHO 1993
Substance	carbendazim, purity not specified
Species	mouse, HOE:NMRKf, groups of 100–120 ♂, 100–120 ♀
Administration route	with the diet
Concentration	0, 50, 150, 300, 1000–5000 mg/kg diet (doses of 0, 5.8/7.1, 17.1/21.2, 34.4/41.9, 548/682 mg/kg body weight and day, ♂/♀ respectively)
Duration	96 weeks
Toxicity	548/682 mg/kg body weight and day: liver toxicity, no influence on mortality

		Dose (mg/kg body weight and day)				
		0	5.8/7.1	17.1/21.2	34.4/41.9	548/682
Tumours and preneoplastic lesions						
Liver						
clear cell foci	♂	0/97	0/99	0/99	0/95	3/99 (3%)
	♀	0/98	0/98	0/95	0/95	4/95 (4%)
basophilic foci	♂	0/97	0/99	1/99 (1%)	0/95	0/99
	♀	0/98	1/98 (1%)	0/95	0/95	0/95
adenomas	♂	3/97 (3%)	2/99 (2%)	0/99	0/95	1/99 (1%)
	♀	0/98	0/98	0/95	1/95 (1%)	0/95
haemangiomas	♂	0/97	2/99 (2%)	3/99 (3%)	2/95 (2%)	0/99
	♀	0/98	0/98	0/95	2/95 (2%)	1/95 (1%)

Table 2 (Continued)

		Dose (mg/kg body weight and day)				
		0	5.8/7.1	17.1/21.2	34.4/41.9	548/682
Lungs						
adenomatosis	♂	29/97 (29%)	31/99 (31%)	30/99 (30%)	25/95 (26%)	22/99 (22%)
	♀	10/98 (10%)	8/98 (8%)	10/95 (11%)	9/95 (9%)	12/95 (13%)
adenocarcinomas	♂	0/97	0/99	0/99	0/95	1/99 (1%)
	♀	0/98	1/98 (1%)	0/95	0/95	0/95
keratinizing squamous cell carcinomas	♂	0/97	0/99	0/99	0/95	0/99
	♀	0/98	0/98	1/95 (1%)	0/95	0/95
cavernous haemangiomas	♂	0/97	0/99	0/99	0/95	0/99
	♀	0/98	1/98 (1%)	0/95	0/95	0/95

Author	JMPR 1995 a; WHO 1993
Substance	carbendazim, purity 50–70%
Species	rat, CD, groups 30 ♂, 30 ♀
Administration route	with the diet
Concentration	0, 100, 500, 5000, 2500–10^000 mg/kg diet (doses of about 0, 3, 15, 150, 300 mg/kg body weight and day)
Duration	104 weeks
Toxicity	150 mg/kg body weight and day and above: ♀: relative liver weights increased, body weight gains decreased

Tumours and preneoplastic lesions

no increased tumour incidences

Author	JMPR 1995 a; WHO 1993
Substance	carbendazim, purity 99%
Species	rat, Wistar, groups of 60 ♂, 60 ♀, only 20 from each group and sex investigated histopathologically
Administration route	with the diet
Concentration	0, 150, 300, 2000–10 000 mg/kg (doses of about 0, 7.5, 15, 500 mg/kg body weight and day)
Duration	2 years
Toxicity	500 mg/kg body weight and day: ♀: haemoglobin decreased, body weight gains decreased, relative liver weights increased, ALAT increased, proliferation of parafollicular thyroid cells increased

Tumours and preneoplastic lesions

no increased tumour incidences

the strain which developed no liver tumours with carbendazim. In two studies with rats, the tumour incidences were not increased after doses of about 300 and 500 mg/kg body weight and day.

The carcinogenicity studies with the metabolic precursor benomyl present a similar picture: also here liver tumours occurred in 2-year feeding studies at the low dose of 64 mg/kg body weight and day and above (500 mg/kg diet) in CD-1 mice, but not in Charles River albino rats up to the highest dose tested of 109 mg/kg body weight and day (2500 mg/kg diet) (JMPR 1995 b).

6 Manifesto (MAK value, classification)

The critical effects of carbendazim are spermatotoxicity and toxic effects on development and the liver.

Carcinogenicity

In two strains of mouse (CD-1 and Swiss) with high incidences of spontaneous liver tumours, carbendazim increased the occurrence of liver tumours and liver hyperplasia. In another strain of mouse (NMRKf), however, no liver tumours were observed at higher doses. Also in two studies with rats, the incidence of liver tumours was not increased after carbendazim doses of 300 and 500 mg/kg body weight and day, respectively. Carbendazim has aneugenic effects, but does not react directly with DNA. An aneugenic effect as the cause of the liver tumours is not plausible on account of the selective effects in only two strains of mouse. The liver tumours are more likely to be connected with tumour promotion, possibly mediated via liver toxicity, as only in mouse strains with a high incidence of spontaneous liver tumours did carbendazim cause an increase in the incidence of liver tumours. The same applies also for benomyl, the metabolic precursor of carbendazim. Carbendazim is therefore not classified in one of the carcinogenicity categories.

MAK value

The findings in CD-1 and Swiss mice are not included in the evaluation because of the specific effects on the liver in these two strains. The NOAELs from long-term feeding studies are 34 mg/kg body weight for NMRKf mice, 15 mg/kg body weight for rats and 4 mg/kg body weight for dogs. Oral absorption in rats is at least 80%; therefore, for mice and dogs oral absorption is assumed also to be 80%. Toxicokinetic extrapolation of these NOAELs to a concentration in air must take into account the daily exposure of the animals compared with the 5-day exposure per week at the workplace (7/5), the species-specific correction values for the toxicokinetic differences between each of the three species and humans of 1:7, 1:4 and 1:1.4, oral absorption of 80%, a body weight of 70 kg, an inhaled air volume of 10 m^3 and the absorption of 100% of the airborne substance via inhalation for humans. The corresponding concentrations are 38, 30 and 22 mg/m^3. As investigations with three animal species yielded very similar concentrations, humans should

not be much more sensitive. The MAK value is set at half the value of the lowest of these concentrations, and using the "preferred value approach, a MAK value of 10 mg/m^3 for the inhalable fraction of carbendazim has, therefore, been established.

Peak limitation

Because of its systemic effects, carbendazim is classified in Peak Limitation Category II. In rats, a half-life of 6.15 hours was determined for the β phase in the target organ liver. The half-life in humans is not known. An excursion factor of 4 has been provisionally set in view of the relatively long half-life in rats.

Prenatal toxicity

In several studies with Sprague Dawley rats, malformations were observed at 19.1 mg/kg body weight and above. On the basis of a NOAEL of 8 mg/kg body weight and day (Sitarek 2001), an inhalation concentration of 11 mg/m^3 is obtained for humans after conversion as above (without correction for 5 days exposure at the workplace). Prenatal toxicity cannot, therefore, be excluded even if the MAK value is observed. Carbendazim is therefore classified in Pregnancy Risk Group B.

Sensitization

There are no findings in humans or results from animal studies that provide evidence that carbendazim causes contact or airway sensitization. Carbendazim is therefore not designated with "Sa" or "Sh".

Dermal absorption

A study with volunteers yielded a value for epicutaneous absorption of 2.2 mg, calculated for an area of skin of 2000 cm^2 and exposure for one hour. Compared with the absorption of 100 mg via inhalation after exposure at the level of the MAK value, the contribution of dermal absorption to systemic toxicity is negligible. Carbendazim is therefore not designated with an "H".

Germ cell mutagenic effects

Carbendazim has aneugenic, but not mutagenic effects. A NEL of 50 mg/kg body weight was calculated for aneuploidy in the mouse. After conversion as described above, this corresponds to 40 mg/m^3. Therefore, if the MAK value is observed, only a very slight contribution to the genetic risk is expected for humans, and carbendazim is classified in Category 5 for Germ Cell Mutagens.

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