# Benomyl

(CAS No: 17804-35-2)

Health-based Reassessment of Administrative Occupational Exposure Limits

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

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# 1 Introduction

The present document contains the assessment of the health hazard of benomyl by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by N Smits M.Sc. (Wageningen University and Research Centre, Wageningen, the Netherlands)*.

The evaluation of the toxicity of benomyl has been based on reviews published by the American Conference of Governmental Industrial Hygienists (ACG99) and in the 'Handbook of Pesticide Toxicology' (Edw91). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in December 1999, literature was searched in the on-line databases Medline, Toxline, and Chemical Abstracts, covering the period 1964-1966 until December 1999, and using the following key words: benomyl and 17804-35-2. Data of unpublished studies were generally not taken into account. Exceptions were made for studies that were summarised and evaluated by international bodies such as the World Health Organization and the Food and Agricultural Organization/World Health Organization (FAO/WHO: Joint Meeting of the FAO Working Party of Experts and the WHO Expert Committee on Pesticide Residues (JMPR) (FAO96), the International Programme on Chemical Safety/World Health Organization (IPCS/WHO) (WHO93a, WHO93b), and the Health Effects Division (HED) of the US Environmental Protection Agency (EPA) as part of its hazard identification assessment review (Sme01). The final literature search was carried out in Medline and Toxline in September 2003.

In October 2003, the President of the Health Council released a draft of the document for public review. No comments were received.

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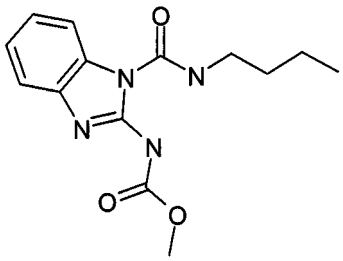
## 2 Identity

| | | |
|---|---|---|
| name | : | benomyl |
| synonyms | : | methyl 1-[1-(butylamino)carbonyl]-1H-benzimidazol-2-ylcarbamate; carbamic acid, [1-(butylamino)carbonyl]-1H-benzimidazol-2-yl]-, methyl ester; methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate; Benlate, Tersan, Fungicide 1991, Fundazol |
| molecular formula | : | $C_{14}H_{18}N_4O_3$ |
| structural formula | : |  |
| CAS number | : | 17804-35-2 |

## 3 Physical and chemical properties

| | | |
|---|---|---|
| molecular weight | : | 290.36 |
| boiling point | : | decomposes just after melting |
| melting point | : | 140ºC |
| flash point | : | not available |
| vapour pressure | : | at 25ºC: <5.0 x $10^{-6}$ Pa |
| solubility in water | : | at 25ºC and pH 5: 3.6 mg/L |
| log $P_{octanol/water}$ | : | 2.12 (experimental); 2.24 (estimated) |
| conversion factors | : | not applicable |

Data from: NLM02, WHO93a, http://esc.syrres.com.

Benomyl is a white crystalline solid, with a faint acrid odour (NLM02).

## 4 Use

Benomyl is a systemic fungicide belonging to the class of benzimidazoles. It has been registered in more than 50 countries for the use on more than 70 crops, including cereals, cotton, grapes, bananas and other fruits, ornamentals,

plantation crops, sugar beet, soybeans, tobacco, turf, vegetables, mushrooms, and many other crops. It can be used under almost all climatic conditions. Benomyl is formulated as a wettable powder (WP) and dry flowable or dispersible granules. In some countries, the latter formulation is no longer available (WHO93a).

According to the database of the Dutch Pesticide Authorisation Board (CTB)*, benomyl is at present not permitted in the Netherlands for use as an active ingredient in pesticides. In the USA, given that production ceased in 2001 and sale and distribution of benomyl products ended on December 31, 2002, US EPA** expected that any use of benomyl-containing products would end in 2003.

## 5 Biotransformation and kinetics

*In vivo*

Blood levels of benomyl and its metabolites carbendazim (MBC) and methyl (5-hydroxy-1H-benzimidazol-2-yl)-carbamate (5-HBC), ranging from 0.25 to 2.3 mg/L, were measured in male rats following inhalation exposure to 320 or 3300 mg/m$^3$ for 0.5 to 6 hours. The main urinary metabolite was identified as 5-HBC (Tur79). A dermal absorption study was conducted in male ChR-CD rats (n=4/group/exposure time), using benomyl in the form of Benlate (50% WP). The test material was applied at dose levels of 0.2, 2, 20, or 200 mg of Benlate, equivalent to 0.1, 1, 10, or 100 mg of [2-$^{14}$C]-benomyl. The exposure durations were 0.5, 1, 2, 4, and 10 hours. Blood concentrations of benomyl and its metabolites carbendazim (MBC) and 5-HBC peaked at 2-4 hours. The amount of benomyl absorbed ranged from 0.031 to 3.5 % from the highest to the lowest dose, respectively, following the maximum exposure period of 10 hours (Bel79).

In a metabolism and tissue distribution study, one rat was fed benomyl for 12 days at a dose level equivalent to 125 mg/kg bw/day, and then received a single oral dose of 7.7 mg [2-$^{14}$C]-benomyl. After 72 hours, 99% of the radioactivity had been recovered, mainly in the urine (85.8% of the administered dose), with 13.1%, 0.2%, and 0.02% in the faeces, the liver and the gastrointestinal tract, and the carcass, respectively. The main urinary metabolite was 5-HBC (Gar74). In a more recent study, rats (n=5/sex/group) were given oral (gavage) doses of 50 or 1000 mg/kg bw [phenyl (U)-$^{14}$C]-carbendazim, the primary metabolite of benomyl. A third group of rats (n=5/sex) was fed 50 mg/kg bw of carbendazim

* At: http://www.ctb-wageningen.nl.
** At: http://www.epa.gov/pesticides/reregistration/status.htm.

for 14 days, and then received a single oral dose of 50 mg/kg bw of radiolabelled carbendazim. In all groups, more than 98% of the radioactivity was recovered within 72 hours after dosing. Urinary excretion accounted for 54-66% or 41% of the administered dose in animals given the low or high dose, respectively. Elimination in the faeces accounted for all of the remaining radiolabel. The amount of radiolabel retained in tissues was less than 1% of the applied dose. The major urinary metabolites were the sulphate conjugate of 5-HBC (21-43% of dose) in the male rats and [2-[(methoxycarbonyl)amino]-6-oxo-6H-benzimidazol-5-yl] β-D-glucopyranosiduronic acid-N-oxide (5,6-HOBC-N-oxide-G; 10-19% of dose) in the female rats. Unchanged carbendazim represented 10-15% of the dose in the faeces of rats in the high-dose group (Mon90).

*In vitro*

An *in vitro* study on the penetration of a 50% WP formulation through the human skin showed that absorption at recommended spray strength solution is poor. Even less penetration was detected when dry concentrated benomyl was applied. No quantitative data were reported (War92).

## 6 Effects and mechanism of action

### Human data

Occupational exposure to benomyl may occur by inhalation of dusts and by skin contact with dusts, emulsions, sprays, and sprayed products. In a field study, maximal dermal and respiratory exposure occurred during loading and mixing for aerial application. Essentially all of the exposure was dermal, resulting in 26 mg per mixing cycle, while the average respiratory exposure was 0.08 mg (duration of exposure not given) (Eve82).

Inhalation of dust may result in irritation of the nose, throat, and respiratory tract. Benomyl can be mildly irritating to the skin and the eyes (Bur93). The chemical caused occasionally contact dermatitis and dermal sensitisation in agricultural workers (Joo83, Küh85, Lis86). A relatively high incidence of contact dermatitis has been reported in women who worked in warm, moist greenhouses where benomyl had been repeatedly sprayed (ACG99).

No poisoning cases with benomyl of agricultural or factory workers have been documented in the scientific literature (Edw91, Gou83). Factory workers engaged in the manufacture of benomyl did not show statistically significant

differences in white and red blood cell count or haemoglobin and haematocrit levels compared to controls. No exposure data were reported (Eve79).

No chromosomal aberrations were found in peripheral lymphocytes of 14 spaymen, using Fundazol 50 WP in greenhouses for up to 2 years (Des90).

Normal birth rates were observed in spouses of male workers (n=298), who were engaged in the manufacture of benomyl for up to 8 years. Spermatogenesis in the workers was not examined, and no exposure levels were reported (Goo78). Levels of inhalation exposure to benomyl and carbendazim experienced by the workers during manufacture of benomyl over the period 1986-1989 were less than 0.3 mg/m$^3$ (WHO93a). A possible case of teratogenicity of Benlate DF has been reported of a woman who delivered a boy without eyes. While pregnant, the woman had had contact with benomyl, while walking in a tomato and strawberry field, previously sprayed with the formulation (Fra98).

## Animal data

*Irritation and sensitisation*

Technical-grade benomyl was slightly irritating to the eyes of rabbits, when tested as a 50% WP and as a suspension in mineral oil. Temporary mild conjunctival irritation and minor transitory corneal opacity were shown after 48 to 96 hours (Fra72). In a later study, similar results were obtained with Benlate PNW, another 50% WP (Gar83a). No dermal irritation was observed 4 or 24 hours after application of 0.5 g of Benlate 50 DF (containing 50% benomyl) to 6 New Zealand white rabbits. After 48 hours, 2 rabbits had slight to mild irritation, which was still evident after 72 hours (Vic87). In another study, application for 24 hours of aqueous suspensions containing 10, 25, or 50% dilutions of technical-grade benomyl on intact and abraded skin of guinea pigs resulted in mild irritation (ACG99, FAO96). Benlate PNW (50% benomyl) was a skin sensitiser in albino guinea pigs, when challenged with 8 or 80% Benlate PNW after 4-weekly intradermal injections of a 0.1% solution in saline (Gar84). In another study, technical-grade benomyl produced sensitisation in all 10 guinea pigs tested in a maximisation test (Mat81).

*Acute toxicity*

Results of acute lethal toxicity of benomyl (as 50% WP) are summarised in Table 1.

*Table 1* Summary of acute toxicity studies for benomyl (as 50% WP) in mammals.

| exposure route (duration) | | species | $LC_{50}/LD_{50}$ | reference |
|---|---|---|---|---|
| inhalation | (4 h) | rat | >820 mg/m$^3$ | Hor69 |
| | (4 h) | rat | >4010 mg/m$^3$ | Bus68a |
| | (4 h) | dog | >1650 mg/m$^3$ | Lit69 |
| dermal | | rabbit | >10000 mg/kg bw | Bus68b |
| | | rabbit | >2000 mg/kg bw | Bro87, Gar83b |
| oral | | rat | >10,000 mg/kg bw[a] | She69a |
| | | rat | >1000 mg/kg bw | She69a |
| | | rat | >1200 mg/kg bw | Hos77 |
| | | rat | >5000 mg/kg bw | Sar87 |
| | | rabbit | >3400 mg/kg bw | Fri69 |
| | | dog | >1000 mg/kg bw[a] | She69b |

[a] Administered as technical-grade benomyl (purity: >95%).

The clinical signs of toxicity after treatment were generally non-specific. Gross and microscopic examination performed in some of these studies revealed testicular degeneration, necrosis of germinal epithelium, and aspermatogenesis in rats and dogs (no doses presented) (FAO96).

Male dogs (n=10/group) were exposed by inhalation to 0, 650, or 1650 mg/m$^3$ for 4 hours, and 5 animals/group were killed at post-exposure days 14 and 28, respectively. Apart from an effect on spermatogenic activity on day 14 (see Section 'Reproduction toxicity'), there was only a significantly decreased liver weight in dogs of the high-concentration group sacrificed at day 28 (Lit69).

In an acute neurotoxicity study, Sprague-Dawley rats (n=10/sex/group) received single oral (gavage) doses of 0, 500, 1000, or 2000 mg/kg bw benomyl (purity: 97.4%). Functional and motor activity were assessed during the week before the day of treatment and at 2 hours, one day, and 7 and 14 days after dosing, after which rats were killed and grossly examined. Histological examination was limited to central and peripheral nervous system tissues from 6 control and all high-dose rats. No treatment-related mortality was observed. Effects noted were a transient reduction in body weight gain and in food intake in all treated groups. Motor activity was reduced, only on the day of treatment, in high-dose females. No abnormalities were recorded in the battery of functional neurotoxicity tests, including reactivity, excitability, gait, sensorimotor coordination, forelimb and hind limb grip strength, abnormal clinical signs, and body temperature, or in the post-mortem examinations. It was concluded that benomyl did not induce acute neurotoxicity at doses up to 2000 mg/kg bw (Fos93).

*Subacute and subchronic toxicity*

In an inhalation study, Sprague-Dawley rats (n=20/sex/group) were exposed nose-only to benomyl aerosols at concentrations of 0, 10, 50, or 200 mg/m$^3$, 6 hours/day, 5 days/week, for approximately 14 weeks. The mass median aerodynamic diameters (MMAD) were in the range of 1.7 to 2.0 µm at 10 and 200 mg/m$^3$, with greater than 93% of all particles being less than 10 µm in aerodynamic diameter. Therefore, an adequate concentration of benomyl reached the lungs. No compound-related mortality was reported. In the high-concentration group, effects observed were decreased mean body weights and reduced food consumption in male rats and reduced body weight gains in male (days 2-37 only) and female rats (days 64-92 only). No significant differences were observed in haematology, clinical chemistry, and urinalysis results between exposed and control animals. Ophthalmoscopic examination did not reveal lesions related to benomyl exposure. After 45 days, 10 animals/sex/group were sacrificed for macroscopic and microscopic examination. A compound-related minimal to mild degeneration of the olfactory epithelium was observed in the nasal cavity of all males and of 8 out of 10 female rats exposed 200 mg/m$^3$. In 2 males exposed to 50 mg/m$^3$, less severe olfactory degeneration was observed. At the end of the approximate 90 days of exposure, all rats exposed to 200 mg/m$^3$ and 3 males exposed to 50 mg/m$^3$ showed minimal to mild degeneration of the olfactory epithelium. Testicular lesions were observed in 2 rats in the high-concentration group. However, only the quantity of spermatids was affected, and no abnormalities were observed in spermatogenesis and in maturation to spermatozoa. Furthermore, the effect observed was unilateral and compatible with a prior unilateral fluid alteration in efferent ducts. Therefore, the lesions were not considered to be a toxic, compound-related effect on spermatogenesis. No other compound-related organ or tissue lesions, including lungs, testes, epididymides, prostate, and seminal vesicle, were observed in any rat at any exposure level. Based on olfactory degeneration in the nasal cavity, the NOAEL was 10 mg/m$^3$ for male rats and 50 mg/m$^3$ for female rats (War89).

In a dermal study in New Zealand white rabbits (n=5/sex/group), a 50% benomyl formulation was applied to abraded dorsal skin at doses equivalent to 0, 50, 250, 500, 1000, and 5000 mg/kg bw/day active ingredient, 6 hours/day, 5 days/week, for 3 weeks. Moderate skin irritation was reported for all dose groups. At the 2 highest dose levels, body weight gains were reduced and diarrhoea, oliguria, and haematuria were observed, indicating functional disturbances of the alimentary tract and the kidneys. At 500 and 1000 mg/kg bw, decreased absolute and relative testes weights were observed. This was not seen

at 5000 mg/kg bw, probably because of the small number of animals examined (n=2). The decrease at 500 mg/kg bw was not considered of biological significance. No histological changes were observed apart from focal testicular degeneration in one rabbit in the 5000-mg/kg bw group. The NOAEL was 500 mg/kg bw/day, based on biologically significantly decreased absolute and relative testes weights (Hoo69).

Sprague-Dawley rats (n=16/sex/group) were fed benomyl (purity: 72%) via the diet, at dose levels equivalent to 0, 5, 25, and 125 mg/kg bw/day, for 96-103 days. No signs of intoxication or treatment-related effects on body weight gain, haematological, liver function (plasma alkaline phosphatase and ALAT), or urine parameters were observed. Females in the high-dose group showed an increase in relative liver weight. Gross and microscopic examination of tissues and organs did not reveal treatment-related abnormalities (She67).

In a neurotoxicity study, functional and motor activity were assessed in Sprague-Dawley rats (n=11/sex/group), fed benomyl (purity: 97.4%) at dietary doses equivalent to 0, 5, 125, or 375 mg/kg bw/day, for 90 days. There was no treatment-related mortality during the study. At the high-dose, body weight gain and food intake were reduced and motor activity was increased. No changes were noted in functional activity, including reactivity, excitability, gait, and sensorimotor coordination. Microscopic examination of tissues from the central and peripheral nervous system did not reveal treatment-related abnormalities. The NOAEL was 25 mg/kg bw (Fos94).

Beagle dogs (n=4/sex/group) were fed benomyl (50% WP) at dietary dose levels equivalent to 0, 2.5, 12.5, and 62.5 mg/kg bw/day (active ingredient), for 3 months. No mortality, signs of intoxication, or effects on growth, food consumption, or urine parameters were observed. There were no dose-related effects on haematological parameters, but in the high-dose group, plasma alkaline phosphatase and ALAT activities were increased and plasma albumin:globulin ratio was significantly decreased in both sexes. In addition, increases in thyroid weights and decreases in thymus weights were also noted in both sexes. One female in the high-dose group had an enlarged spleen, which was consistent with the decreased erythrocyte count, haemoglobin concentration, and haematocrit values. Microscopic examination revealed myeloid hyperplasia of the spleen and bone marrow and erythroid hyperplasia in the same animal. Three males in the high-dose group had reduced prostate weights in comparison with controls. Microscopic examination of tissues and organs showed no consistent effect. The NOAEL was 12.5 mg/kg bw/day (She68).

In another study, conducted by the same author, beagle dogs (n=4/sex/group) were fed benomyl (50% WP) at dietary dose levels equivalent to 0, 2.5, 12.5, and

62.5 mg/kg bw/day (active ingredient), for 2 years. There were no treatment-related effects on mortality and no signs of intoxication were observed. Haematological and urine parameters in treated groups were similar to those of the controls. In the male and female animals of the high-dose group, there were decreased body weight gain, food consumption, and plasma albumin:globulin ratio and increased plasma alkaline phosphatase and ALAT activities - generally more marked in males than in females - while an increase in plasma cholesterol and a decrease in total protein levels were seen in males only. The clinical chemistry observations indicated adverse effects on the liver, characterised as cirrhosis. Four out of 6 dogs in the high-dose group had slight-to-marked bile duct proliferation. No hepatocyte vacuolation was observed at 2.5 or 12.5 mg/kg bw/day, indicating that benomyl is not hepatotoxic at these doses. Focal testicular degeneration was seen in all groups, including controls, with marked testicular degeneration (reduced testicular weight, absence of spermatozoa, and spermatic giant cells) in one out of 3 dogs at 65 mg/kg. The NOAEL was 12.5 mg/kg bw/day, based on effects on the liver, clinical chemistry parameters, and body weight gain at 62.5 mg/kg bw/day (She70).

The effect of benomyl on hepatic enzymes was studied in male and female CrL-CD mice and CD-1 rats. Animals were fed benomyl at dietary doses equivalent to 0, 10, 30, 100, 300, 1000, or 3000 mg/kg bw/day, for 28 days. Significantly elevated liver weight was found in females in the high-dose group, but no liver toxicity or effect on body weight was observed. Enzyme induction of epoxide hydrolase was observed at the 2 high-dose levels and of glutathione *S*-transferase at 3000 mg/kg bw/day in both sexes of mice and rats (Gue81).

The results of short-term toxicity studies in rats, rabbits, and dogs are summarised in Table 2.

*Table 2* Summary of subacute and subchronic studies for benomyl.

| exposure route | species (strain; sex; number) | concentrations/ dose levels | exposure duration | critical effect | NOAEL | reference |
|---|---|---|---|---|---|---|
| inhalation | rat (Sprague-Dawley; n=20/sex/group) | 0, 10, 50, 200 mg/m$^3$ | 14 weeks | degeneration of olfactory epithelium | 10 mg/m$^3$ | War89 |
| dermal | rabbit (New Zealand; n=5/sex/ group) | 0, 50, 250, 500,1000, 5000 mg/kg bw | 3 weeks | changes in testes and bone marrow | 500 mg/kg bw | Hoo69 |
| oral | rat (Sprague-Dawley; (n=16/sex/group) | 0, 5, 25, 125 mg/ kg bw | 14 weeks | increase in liver weight | 25 mg/kg bw | She67 |

| | | | | | |
|---|---|---|---|---|---|
| rat (Sprague-Dawley; (n=11/sex/group) | 0, 5, 125, 375 mg/kg bw | 13 weeks | motor activity | 125 mg/kg bw | Fos94 |
| rat (CD-1) | 0, 10, 30, 100, 300, 1000, 3000 mg/kg bw | 4 weeks | liver enzyme induction | 300 mg/kg bw | Gue81 |
| mice (CrL-CD) | 0, 10, 30, 100, 300, 1000, 3000 mg/kg bw | 4 weeks | liver enzyme induction | 300 mg/kg bw | Gue81 |
| dog (beagle; n=4/sex/group) | 0, 2.5, 12.5, 62.5 mg/kg bw | 3 months | abnormal liver function | 12.5 mg/kg bw | She68 |
| dog (beagle; n=4/sex/group) | 0, 2.5, 12.5, 62.5 mg/kg bw | 2 years | effects on liver | 12.5 mg/kg bw | She70 |

*Chronic toxicity and carcinogenicity*

Groups of Charles River albino weanling rats (n=36/sex/group) were given benomyl (purity: 50-70%) via the diet at doses equivalent to 5, 25, or 125 mg/kg bw/day, for 104 weeks. No treatment-related mortality, signs of toxicity, or changes in food consumption, body weights, or absolute or relative organ weights, or in haematological, liver function, or urine parameters were observed. Upon gross and microscopic examination, no treatment-related neoplastic or non-neoplastic lesions were found in any of the treated groups. As a high incidence of testicular degeneration was observed in control males, no conclusion could be drawn about compound-related effects on male gonads.

The NOAEL was 125 mg/kg bw/day, the highest dose tested (Lee77). Groups of CD-1 mice (n=80/sex/group) were given benomyl (purity: 99%) via the diet at doses equivalent to 0, 75, 225, or 750 mg/kg bw/day, for 2 years. Mice in the high-dose group initially (first 37 weeks) received 1125 mg/kg bw/day. No treatment-related mortality or signs of toxicity were observed. At the mid- and high-dose levels, there were decreases in body weights in both sexes and in haematological parameters and absolute and relative liver weights in males. Except for slight decreases in erythrocyte counts in females of the high-dose group and in haemoglobin, haematocrit, and erythrocyte values in males of the mid-dose group, no remarkable changes were seen in the results of haematological examinations. The most significant changes in organ weights included decreased absolute and relative liver weights in high-dose females and mid- and high-dose males as well as reduced absolute testicular weights in high-dose males. The incidence of hepatocellular carcinomas and benign hepatic neoplasms was significantly increased in a dose-related fashion in treated females and in the low- and mid-dose male groups, but not in the high-dose

males. The latency for liver tumour induction was not different between treated and control groups. The incidences of lung tumours were significantly increased - being within the range of historical controls - in males, but not in females, at the mid and high dose. Non-neoplastic changes observed were confined to the liver (degeneration, pigment, cytomegaly), the thymus (atrophy), and the testes, epididymides, and prostate (degeneration of seminiferous tubules, atrophy, aspermatogenesis, distended ascini) in high-dose males, to the spleen (haemosiderosis) in high-dose females, and to the trachea (submucosal lymphocytic infiltration) in mid-dose females. A NOAEL could not be identified in this study (Wie82).

Several carcinogenicity studies in different strains of mice were conducted with carbendazim, the primary metabolite of benomyl. Like benomyl, carbendazim induced liver tumours (hepatocellular carcinomas and adenomas) in both CD-1 and SPF Swiss mice. In contrast, there was no evidence of a carcinogenic effect from carbendazim when administered in the diet of NMRKf (SPF71) mice (WHO93b).

*Mutagenicity and genotoxicity*

Mutagenicity assays comprised tests for the detection of gene mutations in bacteria (*in vitro*), in mammalian cells (*in vitro*), and in Drosophila, cytogenicity in mammalian cells (*in vitro* and *in vivo*), e.g., sister chromatid exchanges (SCE), structural chromosomal effects (chromosome aberrations and micronucleus formation), numerical chromosomal effects (e.g., aneuploidy), and dominant lethal mutations (*in vivo*), and other genotoxicity assays, e.g., tests for DNA damage (*in vitro*).

- *In vitro* tests:
  - Gene mutation assays. Benomyl did not induce reverse mutations in several strains of *S. typhimurium* (TA98, TA100, TA1530, TA1535, TA1537, TA1538, TA1950), when tested at concentrations up to 5000 µg/plate, with and without metabolic activation by a rat liver microsomal S9 preparation (Car78, Don81, Fis78, Ric83, Rus78, Shi78). In one study, positive results were found in *S. typhimurium* TA1535, in *E. coli* WP2, and *E. coli* WP2 *uvrA*, but not in *S. typhimurium* TA1538, when tested at benomyl concentrations up to 1 µg/mL (Kap76). In *A. nidulans*, benomyl was mutagenic when tested at concentrations up to 0.4 µg/mL (Kap74). In a host-mediated assay in rats or mice given benomyl as a single oral dose of 4000 mg/kg bw, no gene mutations were induced in *S. typhimurium*

TA1950 (Fis78). Negative results were also obtained in *S. typhimurium* G46 $his^-$, in host-mediated assays in mice given benomyl as a single intraperitoneal injection of 2000 mg/kg bw (Shi78) or as 3 consecutive subcutaneous injections of 500 mg/kg bw each (Fis78).
Benomyl (purity: 99.9%) did not induce gene mutations in the HPRT forward mutation assay in cultured Chinese hamster ovary cells (CHO) when tested at concentrations of 17 or 172 µmol/L in the absence or 3 or 120 µmol/L in the presence of S9 activation (Fit80). The $TK^{+/-}$ mutation assay in cultured L5178Y mouse lymphoma cells was negative at benomyl concentrations up to 20 µmol/L without or 25 µmol/L with metabolic activation (Ama79). Negative results in the mouse lymphoma L5178Y assay were also obtained in an unpublished study, when benomyl was tested at concentrations up to 25 µmol/L in the absence of metabolic activation. However, in the presence of S9, an increased gene mutation rate was found (McC83), while in a third study, results were positive, both with and without metabolic activation, at concentrations in the range of 5 to 100 mg/L (with), and 2 to 40 mg/L benomyl (without) (Jon84).
No sex-linked recessive lethal mutations were found in *D. melanogaster* when fed a suspension of 1 mg benomyl/mL (Lam80).

- Cytogenicity assays. Weakly positive SCE results were seen in cultured CHO cells when tested at benomyl concentrations above 150 mg/L, without metabolic activation (Eva80). In another study, positive results of SCE in CHO cells were observed, with and without metabolic activation, at benomyl concentrations in the range of 3 to 36 mg/L (with S9), or 0.6 to 2.5 mg/L (without S9) (Jon84). The frequency of SCEs in cultured human lymphocytes was slightly increased at concentrations ranging from 1 to 100 mg/L in the absence of S9 (Dol92). When tested in cultured human lymphocytes, the frequency of chromosome aberrations remained unaffected when tested at benomyl concentrations up to 2000 mg/L with and without metabolic activation (Gup75). In another study, an increased chromosome aberration rate was found at 10 mg/L without S9, but not in the presence of S9 (Pil83). Using the same test system, a not statistically significant, not dose-related increase in the frequency of micronuclei was found at concentrations ranging from 1 to 100 mg/L (Bia97). An increase in chromosome aberration rate was observed in benomyl-treated cultured Chinese hamster lung cells, when tested at concentrations up to 90 mg/mL, with and without metabolic activation (Sas88). Using cultured rat hepatocytes, benomyl induced a significant dose-related increase in the frequency of micronuclei (concentration range tested: 0.5-25 µg/mL)

(Pia94).
In several studies, a dose-related increase in numerical chromosomal aberrations was observed in benomyl-treated cell lines. Aneuploidy and/or polyploidy was observed in human/mouse monochromosomal hybrid cells at concentrations in the range of 1.5 to 15 mg/L (Ath85), in V79/AP4 Chinese hamster cells (Rai89), in CHO cells (Eas89), in human lymphocytes, at concentrations above 0.1 (Geo90) or 1 mg/L (Ben00), and in Chinese hamster/human hybrid cells, at concentrations above 2.0 mg/L (Zel90).
When fed at a suspension of 1 mg/mL, benomyl did not significantly increase non-disjunction, chromosome breaks, or chromosome loss in *D. melanogaster*. However, there was a relatively high incidence of sterility, which could be expected from a spindle inhibitor (Lam80).

- Other assays. The rec-assay for DNA repair was negative in *B. subtilis* at 2000 μg benomyl/plate (Shi78). Benomyl did not increase unscheduled DNA synthesis in rat and mouse hepatocytes at concentrations in the range of 0.5 to 500 mg/L (Ton81). Benomyl did not induce non-disjunction in *A. nidulans* when tested at concentrations up to 2.8 mmol/L. However, spindle inhibition was observed (DeB80).

- *In vivo* tests:
  - No dominant lethal mutations were induced in ChR-CD rats, given benomyl via the feed at doses equivalent to 0, 25, 125, or 250 mg/kg bw/day, for 7 days (She75). Negative results were also found in other studies, when Wistar rats were given benomyl via the feed at doses equivalent to 0, 0.05, 0.31, or 10 mg/kg bw/day for 70 days (Bar83) or by gavage at daily doses of 0, 10, or 50 mg/kg bw for 70 days (Geo90). Benomyl, orally (gavage) administered at single doses of 0, 500, 1000, 1500, 1750, or 2000 mg/kg bw, induced an increased frequency of hyperploid cells in oocytes collected from superovulated female ICR mice (n=20-59/group) 17 hours after administration, at all doses. Treatment did not cause an increase in structural chromosome aberrations (Mai92). When male (probably Wistar) rats (n=4-5/group) were orally given doses of Fundazol (containing 80% benomyl as active ingredient) 0, 10, 50, or 200 mg/kg bw, for 70 days, a small (1.7 fold over control values), statistically significant increase in the frequency of micronuclei was found in the bone marrow of animals sacrificed 24 hours after last administration of 50 mg/kg bw. No effects were seen at 10 or 200 mg/kg bw (Geo90). In ICR mice given 2 oral (gavage) doses of 0, 500, or 1000

mg benomyl/kg bw each, 24 hours apart, a statistically significant increased incidence of micronuclei in polychromatic erythrocytes was observed at the top dose only (Sei76). In another study, an increase in the frequency of micronuclei was observed in bone marrow cells obtained from B6D2F2/Cr-1Br mice (n=5/sex/group) harvested 48 hours after administration of single oral doses of 2500 or 5000 mg/kg bw. Analysis revealed that on average 82% of the total micronucleated polychromatic erythrocytes showed positive kinetochore staining, indicating benomyl-induced aneuploidy. No effects were seen at 100 mg/kg bw (Ben92, Sar94). A single oral dose of 1000 mg/kg bw caused a small but significant induction of micronuclei in the bone marrow cells of male Swiss mice, with a maximum (3.6-fold over control values) at 38 hours after administration. Animals were sacrificed at 5 time points between 6 and 48 hours after administration, and bone marrow samples of at least 4 animals were analysed per time point. Polyploid cells were significantly increased over control levels (at t=0) only at 30 hours whereas some hyperploid cells (not statistically significant) were observed at 38 hours (Bar93). An increased frequency of micronuclei was also found in polychromatic erythrocytes of mice, given oral (gavage) doses of 2500 or 5000 mg/kg bw (Sas90) or intraperitoneally injected with single doses ranging from 250 to1000 mg benomyl/kg bw (Jon84).

No increased incidences of chromosome aberrations were found in bone marrow cells of rats, given benomyl at daily oral (gavage) doses up to 500 mg/kg bw for 8 days (Ruz76). No increase in structural chromosomal aberrations was observed in bone marrow cells of B6D2F2/Cr-1Br mice, given single oral (gavage) doses of benomyl of 625, 1250, 2500, and 5000 mg/kg bw (Sta90). A single dose of 1000 mg/kg bw did not increase the percentage of aberrant cells without gaps analysed in bone marrow cells of male Swiss mice at time points ranging from 6 to 48 hours (n= at least 4 animals/time point) (Bar93). Single oral (gavage) doses of 500 and 1000 mg/kg bw or 5 daily doses of 500 mg/kg bw did not induce an increased frequency of chromosome aberrations in bone marrow cells of male mice (time of sacrifice 20 hours after final administration) (Pil80). Following intraperitoneal injections, twice at intervals of 24 hours, of doses of 250, 500, or 1000 mg/kg bw, a dose-dependent increase in the total breakage frequency and in the frequency of aberrant cells, both being statistically significantly different from controls at the high dose only, was observed in bone marrow cells of male Wistar rats (n=5/group) (Adh88).

- Other tests:
  Using the Syrian hamster embryo cell transformation assay, a test thought to detect carcinogens with known or suspected aneuploidy-inducing activity, benomyl did not induce a significant increase in the frequency of morphologically transformed colonies, when exposed to concentrations ranging from 0.25 to 2.1 mg/L for 7 days. However, exposure for 24 hours caused a significant increase in the transformation frequency at 2 mg/L benomyl (Gib95).

In summary, *in vitro* tests showed that benomyl induced SCEs and numerical chromosomal effects. Results of the induction of gene mutations or structural chromosomal aberrations in benomyl-treated mammalian cells were conflicting.

*In vivo*, benomyl-treated mice had an increased incidence of micronuclei or aneuploidy. Numerical chromosome changes might be a result of the interference of benomyl, and its primary metabolite carbendazim, with cell division (Alb93, Dav77).

*Reproduction toxicity*

Several studies have been conducted on the effects of benomyl on male reproductive organs.

When male dogs (n=10/group) were exposed by inhalation to 0, 650, or 1650 mg/m$^3$ for 4 hours, there was only a slight reduction in spermatogenic activity in the high-concentration group at post-exposure day 14 when half of the animals were killed, but not at day 28 when the remainder of the animals were sacrificed (Lit69). In a follow-up study, gross and microscopic changes in testes and epididymides, including spermatocyte degeneration, were seen in male Sprague-Dawley rats exposed to 1900 mg/m$^3$ for 4 hours (no more data presented) (War89). In Sprague-Dawley rats (n=20/group) exposed to 10, 50, or 200 mg/m$^3$, 6 hours/day, 5 days/week, for 14 weeks, no compound-related effects on male reproductive organs or tissue were seen in any of the groups (see also Section ‘Subacute and subchronic toxicity’) (War89).

The effects on the testes following single oral administration were studied in adult Sprague-Dawley rats (n=20/group), by giving doses of 0, 25, 50, 100, 200, 400, or 800 mg/kg bw benomyl (95% active ingredient) in corn oil by gavage. Animals were sacrificed at 2 and 70 days after treatment for microscopic examination. At day 2 after treatment, testicular swelling, occlusions of the efferent ductules, and premature release of germ cells (sloughing) in the tubules were observed at doses of 100 mg/kg bw and above. Testis weight was

significantly increased at 200 mg/kg bw and above. At day 70 after treatment, dose-dependent increases in seminiferous tubular atrophy and in the number of reproductive tracts containing occluded efferent ductules were seen, starting at 50 mg/kg bw. The NOAEL was 25 mg/kg bw, based on biologically significant sloughing and occlusions of the efferent ductules of the testes (Hes91).

In another study, 65-day-old male Sprague-Dawley rats received 10 daily doses of benomyl at 0, 200, or 400 mg/kg bw/day by gavage. There were no compound-related effects on body weights and on the absolute weights of liver, kidney, adrenal, testis, or seminal vesicles. However, treatment-related reductions were observed in caudate epididymal weights, in epididymal sperm count, and in vas deferens sperm concentration. Microscopic examination revealed that at the high-dose, hypospermatocytogenesis with generalised disruption of all stages of spermatogenesis occurred. No NOAEL was established in this study (Car82).

Adult male Wistar rats (n=27/group) were fed benomyl at dietary levels equivalent to 0, 0.1, 0.6, or 20 mg/kg bw/day, for 70 days. At the high-dose, sperm counts in ejaculate were statistically significantly reduced. There was a dose-related decrease in relative testis weights and in fertility index, starting at 0.1 mg/kg bw/day. No change in copular behaviour was noted, and the compound did not induce dominant lethal mutations. Plasma testosterone and gonadotropin levels were not influenced by the treatment. The treatment-related effects were completely reversible, 70 days after the end of dosing. The LOAEL was 0.1 mg/kg bw/day (Bar83). However, the breeding technique used in this study may have compromised the results. Male and female animals did not receive food (to avoid female exposure) over an 8-hour period during the normal dark cycle, apparently for up to 7 or more consecutive days, which may have influenced breeding patterns. Furthermore, these fasting periods may have exacerbated the testicular effects. Fasted rats appeared to be markedly more susceptible to testicular effects induced by a single dose of the benomyl metabolite MBC than non-fasted animals (Lin88).

In another study, 102-day-old Wistar male rats (n=12/group) were given benomyl at 0, 1, 5, 15, or 45 mg/kg bw/day by gavage, for 62 days. They were then bred to untreated females, and sacrificed at days 14-17 after the end of treatment. No differences between treated and control groups were seen in reproductive behaviour parameters (number of copulatory plugs, sperm-positive smears, total litters, pups per litter, and implantation sites per litter, percentage of resorptions per litter, and male and female pup weights), seminal vesicle weight, prostate weight, sperm motility, and in the concentrations of serum testosterone, luteinising hormone (LH), follicle-stimulating hormone (FSH), prolactin, or

androgen binding protein. At the high-dose, decreased testicular and epididymal weights, decreased sperm production, and increased numbers of decapitated spermatozoa and of seminiferous tubules containing multinucleated giant cells were observed. The NOAEL was 15 mg/kg bw/day (Lin88).

In a study performed according to (and meant to evaluate) OECD Guideline 421 (Reproductive Toxicity Screening Test), male and female Wistar-derived (WU) rats (n=10/sex/group) were given oral (gavage) doses of benomyl (in corn oil) of 0, 10, 30, or 90 mg/kg bw/day, for a 14-day pre-mating period and a 14-day (1:1) mating period, after which the males were killed and the females were treated for another 28 days until terminal sacrifice at post-partum day 6. Apart from increases in food consumption and body weight gain in high-dose females during the first 2 treatment weeks, no differences between groups were seen regarding food consumption and body weight. No other treatment-related changes were reported in parental animals during the exposure period. Upon post-mortem examination, sex organs were not affected in females. In males, there were no changes in testes and epididymides weights, but a dose-dependent increase of incidence and severity of testicular epithelial degeneration was seen, becoming significant in the high-dose group. Mating resulted in 8, 8, and 4 pregnancies in the low-, mid-, and high-dose group, respectively, vs. 5 in the control group. There were no differences in time to conception and pregnancy duration between groups, and all pregnancies resulted in normal birth. Pup weights at birth and on post-natal day 6 were significantly lower in the high-dose group. Pre-implantation and post-implantation losses seemed to be increased in treated animals, but there were no differences between groups in numbers of corpora lutea, pup sex ratio, and post-natal mortality or in incidences of fetal anomalies or malformations. Compared with a concomitant developmental study (see below), no eye malformations or high incidences of prenatal deaths were observed at the high dose of 90
mg/kg bw. Piersma et al. stated that modulation of maternal metabolic enzyme activity during the pre-mating exposure period might have played a role in this effect (Pie95).

In a 2-generation reproduction study, Sprague-Dawley rats (numbers not given) were given benomyl in the diet at levels equivalent to 0, 6, 30, 190, or 350 mg/kg bw/day for 71 days prior to mating, and F1 offspring were fed treated diets at levels of 0, 8, 20, 250, or 1000 mg/kg bw/day for at least 105 days after weaning, before being bred for production of F2a and F2b litters. No treatment-related parental mortality was observed. At the high-dose, F0 and F1 parental body weights, body weight gain, and food consumption were depressed, and

male rats had decreased testicular weights. Sperm count was reduced at 190 and 350 mg/kg bw for F0 parents, and at 250 and 1000 mg/kg bw for F1 parents. Microscopic examination revealed atrophy and degeneration of the seminiferous tubules in the testes and oligospermia in F0 and F1 parents at the 2 high-dose levels. There were no treatment-related changes in mating, fertility, or gestation indices. No abnormalities were noted in the percent of pups born alive. However, at the high-dose, all male and female offspring (F1 and F2) had decreased birth weights and F2a and F2b offspring had significantly decreased viability indices. F2 offspring also had significantly depressed body weights on days 14 and 21 of lactation at 250 mg/kg bw/day. The parental and reproductive NOAEL were 30 and 20 mg/kg bw/day, respectively (Meb90).

Benomyl was evaluated for developmental toxicity in rats, mice and rabbits. In a study with pregnant Wistar rats, the animals (n=27-28/group) were given benomyl (purity: 99%) via the diet, at doses equivalent to 0, 169, 298, or 505 mg/kg bw/day, on days 7-16 of gestation. On day 20 of gestation, all pregnant animals were sacrificed and fetuses delivered by Caesarean section. Food consumption and body weight gain were reduced in dams at the mid- and high-dose levels. At these doses, the weight gain of fetuses was also reduced and the percentage of fetuses with enlarged renal pelvis was increased, compared with animals in the control group. At the high dose, a significant decrease was observed in the ossification of the supraoccipital bone. The developmental and maternal NOAEL was 169 mg/kg bw/day (Kav82).

In another study by the same authors, pregnant rats (n=12-30/group) received benomyl (purity: 99%) at oral (gavage) doses 0, 15.6, 31.2, 62.5, or 125 mg/kg bw/day, on days 7-16 of gestation. Pups were delivered by Caesarean section on day 21 of gestation. A significant reduction in maternal body weight was observed at the highest dose level. The frequency of fetal resorptions and the fetal mortality was increased at 125 mg/kg bw/day. Fetal weight was significantly reduced at 62.5 and 125 mg/kg bw/day. At these levels, there were also increased incidences of skeletal, visceral, and brain malformations, e.g., decreased numbers of sternal and caudal vertebrae, increased percentages of enlarged renal pelvis, increased percentages of enlarged lateral ventricles, hydrocephaly, and microphthalmia. The maternal NOAEL was 62.5 mg/kg bw/day and the developmental NOAEL 31.2 mg/kg bw/day (Kav82).

In a separate study, the authors also evaluated the effect of low levels of benomyl as the pups aged. Groups of pregnant Wistar rats were given benomyl by gavage at doses of 0, 15.6 or 31.2 mg/kg bw/day from day 7 of gestation until delivery, and from delivery to day 22 of lactation. The litters were each reduced

to 4 male and 4 female pups on day 3 of lactation, and weighed on days 8, 15, 22, 29, and 100 after parturition. The locomotor activity was evaluated periodically throughout the study. At 100 days of age, several organs were weighed. No treatment-related effects were noted on litter size at birth or weaning or on body weights of fetuses. Growth, survival, locomotor activity, and organ weights (adrenals, liver, kidneys, ovaries) of offspring were comparable to those of controls. However, weights of testes, ventral prostate, and seminal vesicles were significantly decreased at the high dose. The NOAEL for developmental toxicity was 15.6 mg/kg bw/day (Kav82).

In another developmental toxicity study, benomyl (purity: 99.2%) was given to pregnant Sprague-Dawley rats (n=27/group) at oral (gavage) doses of 0, 3, 10, 30, 62.5, or 125 mg/kg bw/day, on days 7-16 of gestation. No treatment-related maternal effects were observed. However, embryofetal mortality was increased at 125 mg/kg bw and fetal body weight decreased at 62.5 and 125 mg/kg bw/day. Major skeletal malformations were observed at 62.5 and 125 mg/kg bw/day and included misaligned and unossified sternebrae, fused ribs, and thoracic arches. Malformations, including microphthalmia, anophthalmia, and hydrocephaly (distended lateral ventricles) were observed at doses of 10 mg/kg bw/day and above, being dose related at the higher dose levels. Microscopic changes of the eyes were also found at these doses, but not at 30 mg/kg bw/day. The authors considered microphthalmia at 10 mg/kg bw/day to be related to treatment in view of the severity of microscopic changes. The maternal NOAEL was >125 mg/kg bw/day, and the developmental NOAEL 3 mg/kg bw/day (Sta80).

In a follow-up study by the same authors, conducted to determine a no-effect level for external hydrocephaly and microphthalmia, benomyl (purity: 99.1%) was orally (gavage) administered to pregnant Sprague-Dawley rats (n=50/group), on days 7-16 of gestation, at dose levels of 0, 3, 6.25, 10, 20, 30, or 62.5 mg/kg bw/day. Mean fetal body weight was decreased and incidental observations of microphthalmia and hydrocephaly occurred at 62.5 mg/kg bw/day. No other maternal or developmental effects were noted. The maternal NOAEL was >62.5 mg/kg bw, and the NOAEL for microphthalmia 30 mg/kg bw/day (Sta82).

In another study, groups of pregnant Sprague-Dawley rats were treated with 0 or 62.5 mg/kg bw/day by gavage, on days 7-16 (8 rats) or 7-20 of gestation (13 rats). No maternal effects were noted. Hydrocephaly was noted in 35% of fetuses examined on day 16 and in 76% of fetuses examined on day 20 (Ell87, Ell88).

In a comparable study, ocular anomalies (microphthalmia, anophthalmia, retinal dysplasia, cataracts) occurred in 43% of the fetuses of dams (Sprague-

Dawley) given (by gavage) 62.4 mg/kg bw/day of benomyl on days 7-21 of gestation. The NOAEL was 31.2 mg/kg bw/day (Hoo91).

In female Wistar-derived (WU) rats (n=10/group) given oral (gavage) doses of 0, 90, or 270 mg/kg bw on days 6 to 15 of gestation, body weight gain was significantly reduced in both treated groups. In both groups, post-implantation loss and the number of total litter resorptions and of fetuses with ophthalmic abnormalities were increased and the body weights of surviving near-term fetuses decreased. Numbers of corpora lutea and pre-implantation loss did not differ among groups (Pie95).

Groups of pregnant CD-1 mice were given benomyl by gavage at 0, 50, 100, or 200 mg/kg bw/day, on days 7-17 of gestation. No treatment-related maternal effects were observed. A dose-related increase in fetal mortality and a decrease in fetal weight were observed at all levels. The numbers of caudal and sternal ossifications were decreased and the incidences of enlarged lateral ventricles were increased at the high dose. A dose-related increase in the incidence of enlarged renal pelvises was observed at 100 and 200 mg/kg bw/day and an increase in the occurrence of supernumerary ribs at all levels. The numbers of abnormal litters and fetuses were significantly increased at 100 and 200 mg/kg bw/day. Major anomalies included brain defects, e.g., hydrocephaly, exencephaly, and skeletal abnormalities, e.g., fused ribs, fused vertebrae. No NOAEL was established for fetotoxicity and teratogenicity (Kav82).

Groups of pregnant New Zealand white rabbits (n=20/group) were given oral doses of 0, 15, 30, 90, or 180 mg/kg bw/day of benomyl, on days 7-28 of gestation. Maternal mortality, occurring at 0, 30, 90, and 180 mg/kg bw, was not related to benomyl toxicity. At 180 mg/kg bw, food consumption was significantly reduced and 2 animals aborted during the study. Fetal mortality and fetal weights were not affected by the treatment and there were no treatment-related external malformations. However, a treatment-related decrease in renal papillae was observed in 2 viable fetuses in 2 litters. The NOAEL for both maternal and developmental toxicity was 90 mg/kg bw/day (Mun95).

A summary of oral reproductive and developmental toxicity studies with benomyl is shown in Table 3.

*Table 3* Summary of oral reproductive and developmental toxicity studies with benomyl.

| species (strain; number; sex) | dose level (mg/kg bw) | exposure duration | critical effect | NOAEL | reference |
|---|---|---|---|---|---|
| rat (Sprague-Dawley; n=20/males/group) | 0, 25, 50, 100, 200, 400, 800 (gavage) | 1 day | testicular effects; occluded efferent ductules | 25 | Hes91 |
| rat (Sprague-Dawley; males) | 0, 200, 400 (gavage) | 10 days | reduced epididymus weight, sperm count, disrupted spermatogenesis | LOAEL: 200 | Car82 |
| rat (Wistar; n=27 males/ group) | 0, 0.1, 0.6, 20 (diet) | 70 days | reduced testicular weight and fertility index | LOAEL: 0.1 | Bar83 |
| rat (Wistar; n=12 males/ group) | 0, 1, 5, 15, 45 (gavage) | 62 days | reduced testicular weight, epididymal weight, sperm production, and effect on spermatogenesis | 15 | Lin88 |
| rat (WU; n=10/sex/group) | 0, 10, 30, 90 (gavage) | 28 d (males)<br>54 d (females) | paternal: testicular epithelial degeneration<br>maternal: none<br>fetal: decreased pup weight | 30<br>90<br>30 | Pie95 |
| rat (Sprague-Dawley; males, females) | F0: 0, 6, 30, 190, 330 (diet)<br>F1: 0, 8, 20, 250, 1000 (diet) | 71 days<br>105 days | maternal: reduced sperm counts<br>F1/F2: reduced body weight during lactation | 30<br>20 | Meb90 |
| rat (Wistar; n=27-28 females/group) | 0, 169, 298, 505 (diet) | gestational day 7-16 | maternal: body weight<br>fetal: enlarged renal pelvis | 169<br>169 | Kav82 |
| rat (Wistar; n=12-30 females/group) | 0, 15.6, 31.2, 62.5, 125 (gavage) | gestational day 7-16 | maternal: body weight<br>fetal: skeletal, visceral, brain malformations | 62.5<br>31.2 | Kav82 |
| rat (Wistar; females) | 0, 15.6, 31.2 (gavage) | gestational day 7-15 | maternal: none<br>fetal: testicular weight<br>prostate weight | >31.2<br>15.6 | Kav82 |
| rat (Sprague-Dawley; n=27 females/group) | 0, 3, 10, 30, 62.5, 125 (gavage) | gestational day 7-16 | maternal: none<br>fetal: external, visceral anomalies | >125<br>3 | Sta80 |
| rat (Sprague-Dawley; n=50 females/group) | 0, 3, 6.25, 10, 20, 30, 62.5 (gavage) | gestational day 7-16 | maternal: none<br>fetal: microphthalmia | >62.5<br>30 | Sta82 |
| rat (Sprague-Dawley; n=8-13 females/group) | 0, 62.5 (gavage) | gestational day 7-16 or 7-20 | maternal: none<br>fetal: hydrocephaly | >62.5<br>LOAEL: 62.5 | Ell87, Ell88 |
| rat (Sprague-Dawley; females) | 0, 31.2, 62.4 (gavage) | gestational day 7-21 | maternal: none<br>fetal: microphthalmia | >62.4<br>31.2 | Hoo91 |

| | | | | | |
|---|---|---|---|---|---|
| rat<br>(WU; n=10 females/ group) | 0, 90, 270 (gavage) | gestational day 6-15 | maternal: decreased body weight<br>fetal: increases in post-implantation loss, total litter resorptions, fetuses with ophthalmic abnormalities; decreased body weight | LOAEL: 90<br>LOAEL: 90 | Pie95 |
| mouse<br>(CD-1; n=20-25 females/group) | 0, 50, 100, 200 | gestational day 7-17 | maternal: none<br>fetal: skeletal, visceral and brain anomalies | >200<br>LOAEL: 50 | Kav82 |
| rabbit<br>(New Zealand; n=20 females/group) | 0, 15, 30, 90, 180 | gestational day 7-28 | maternal: reduced food consumption; abortions; clinical signs<br>fetal: renal papillae anomalies | 90<br>90 | Mun95 |

In summary, benomyl induced effects on male reproductive organs in rats. Following inhalation, testicular lesions were observed in rats and dogs at single 4-hour exposures to 1900 (only level tested) and 1650 mg/m$^3$, respectively, while no such effects were seen in dogs exposed to 650 mg/m$^3$ for 4 hours or in rats exposed to 200 mg/m$^3$ (highest concentration tested), 6 hours/day, 5 days/week, for 13 weeks. Following single and repeated oral administration, the NOAELs for testicular effects were 25 and 15 mg/kg bw, respectively, and the corresponding LOAELs 50 and 45 mg/kg bw, respectively.

In a 2-generation reproduction toxicity study, the NOAELs for parental and reproductive effects were 30 and 20 mg/kg bw, respectively, based on decreased sperm counts and reduced body weights of F2 pups during lactation.

Developmental toxicity studies in rats showed effects at levels substantially below those causing maternal toxicity, the NOAELs for maternal and developmental toxicity being 62.5 and 15 mg/kg bw, respectively. In mice, fetal anomalies were seen at 50 mg/kg bw, the lowest level tested, while no maternal toxicity was observed at 200 mg/kg bw, the highest level tested. In rabbits, the NOAEL for both maternal and developmental effects was 90 mg/kg bw.

## 7 Existing guidelines

The current administrative occupational exposure limit (MAC) for benomyl in the Netherlands is 10 mg/m$^3$, 8-hour TWA.

Existing occupational exposure limits for benomyl in some European countries and in the USA are summarised in the annex.

## 8 Assessment of health hazard

The health hazard assessment of benomyl is based to a large extent on toxicology reviews issued by the World Health organization/International Programme on Chemical Safety (WHO93a), the FAO/WHO Joint Meeting on Pesticide Residues for recommendation of an acceptable daily intake (ADI) (FAO96), and the Health Effect Division of the United States EPA for reregistration eligibility (Sme01). The toxicology profile in these reviews is obtained mainly from unpublished reports of toxicology studies, conducted for registration purposes.

Workers can be exposed to benomyl through inhalation of dust or aerosols or by direct skin contact with a formulation of the compound. Benomyl was poorly absorbed through human skin *in vitro* (not quantitated) and through rat skin *in vivo*. In rats, the dermal absorption of benomyl (50% wettable powder) ranged from 0.031 to 3.5% and was inversely related to the amount of benomyl applied on the skin. No data is available of the percentage uptake of the compound through the lungs. The extent of absorption following oral intake is 85-90% in rats. Following absorption, the compound was rapidly metabolised into carbendazim, which was further metabolised into a range of breakdown products (e.g., 5-HBC). These metabolites were excreted in the urine and the faeces within 72 hours after application. There is no evidence of accumulation of the compound in any of the tissues.

Case studies in humans showed that benomyl caused contact dermatitis and dermal sensitisation in agricultural workers. No inadvertent systemic poisoning of agricultural or factory workers has been documented. In factory workers, no abnormalities in haematological parameters, fertility, or cytogenicity parameters have been reported, at benomyl concentrations in air that were generally below 0.03 $mg/m^3$.

In experimental animals, the compound is slightly irritating to the eyes and the skin and a potent skin sensitiser.

Based on the results of acute lethal toxicity studies in test animals, the committee considers the compound as unlikely to present an acute health hazard. Four-hour exposure to benomyl concentrations of 1650 or 1900 $mg/m^3$ caused slight testicular effects in rats and dogs, respectively, while single oral doses of 50 mg/kg bw and higher induced effects such as occlusions of the efferent ductules and premature release of germ cells (sloughing) in the tubules. Benomyl did not induce acute neurotoxicity in rats at single oral doses up to 2000 mg/kg bw, apart from a decrease at the treatment day in motor activity in females given 2000 mg/kg bw.

Effects of benomyl exposure in subchronic or chronic studies in rats, mice, dogs, and rabbits included: degeneration of olfactory epithelium in a 13-week inhalation study in rats (NOAEL: 10 mg/m$^3$); testicular changes in a 3-week dermal study in rabbits (NOAEL: 500 mg/kg bw/day); abnormalities in the liver in a 14-week oral study in rats (NOAEL: 25 mg/kg bw/day) and in a 2-year study in dogs (NOAEL: 12.5 mg/kg bw/day); hepatocellular carcinomas in a 2-year study in mice (LOAEL: 75 mg/kg bw/day).

Benomyl was not mutagenic in bacteria, but results of gene mutation and cytogenicity assays in cultured mammalian cells were conflicting. *In vitro*, benomyl induced aneuploidy/polyploidy in mammalian cells. *In vivo*, benomyl did not induce gene mutations in the host-mediated assay, or dominant lethal mutations, but the incidence of micronuclei and aneuploidy in blood cells of mice was increased. Aneuploidy might have been caused by interference of benomyl, or its primary metabolite carbendazim, with cell division. According to the committee, there is inadequate evidence for mutagenicity of benomyl. The committee comments, however, that the *in vivo* host-mediated gene mutation assay and the dominant lethal mutation assay are insensitive tests, which are not used anymore in nowadays mutagenicity testing.

Carcinogenicity study in rats did not show treatment-related increased incidences of neoplastic lesions. In CD-1 mice, however, the incidence of hepatocellular carcinomas was significantly increased in a dose-related fashion in both sexes. The committee considers the relevance of this increase for man as controversial, because of the high incidence of spontaneous liver tumours in this strain of mice.

Subchronic oral studies to examine the effects of benomyl exposure on the reproductive organs in male rats showed the following effects: decreased testicular and epididymal weights and a decreased sperm production with generalised disruption of all stages of spermatogenesis in a 62-day oral study (NOAEL: 15 mg/kg bw/day). In a subchronic inhalation study, no such effects were seen in rats exposed to 200 mg/m$^3$, the highest concentration tested. In a 2-generation reproductive toxicity study in rats, the parental and reproductive NOAELs were 30 and 20 mg/kg bw/day, on the basis of decreased sperm counts in parental males, and reduced body weights of F2 pups, respectively. In developmental toxicity studies, teratogenicity was demonstrated in rats at dose levels below those causing maternal toxicity. The major abnormalities included skeletal, visceral, and brain abnormalities (hydrocephaly and microphthalmia). In one study, offspring had decreased testicular and prostate weights. The committee concluded that the NOAELs for developmental and maternal toxicity were 62.5 and 15 mg/kg bw/day, respectively; in mice, they were ≤50 and ≥200

mg/kg bw/day, respectively. In rabbits, 90 mg/kg bw/day was the NOAEL for both maternal and developmental toxicity.

Based on the above data, the committee takes the well-performed 14-week inhalation study in rats (War89), with a NOAEL of 10 mg/m$^3$, as a starting point in deriving a health-based recommended occupational exposure limit (HBROEL). In this study, the critical effect was degeneration of olfactory epithelium in male animals at concentrations of 50 mg/m$^3$ and above, while no treatment-related effects were seen on male reproduction at 200 mg/m$^3$, the highest concentration tested. For extrapolation to a HBROEL, an overall assessment factor of 8 is established. This factor covers intra- and interspecies variation, and the type of critical effect. Thus, applying this factor and the preferred value approach, a health-based occupational exposure limit of 1 mg/m$^3$ is recommended for benomyl.

The committee considers this HBROEL to be sufficient to protect workers from reproduction toxicity effects. Assuming a 60-70-kg worker inhales 10 m$^3$ during an 8-hour working day and a retention of 100%, this HBROEL would lead to a daily intake of approximately 0.15 mg/kg bw/day, which is a factor of 100 lower than the NOAELs of 15 mg/kg bw/day for developmental and male reproduction toxicity found in rat studies

The committee recommends a health-based occupational exposure limit for benomyl of 1 mg/m$^3$, as inhalable dust, as an 8-hour time-weighted average (TWA).

In view of the low penetration ability of benomyl (when tested as a 50% wettable powder) through human and rat skin, the committee deems a skin notation not necessary.

## References


ACG99 American Conference of Governmental Industrial Hygienists (ACGIH). Benomyl. In: TLVs® and other occupational exposure values - 1999. [CD-ROM]. Cincinnati OH, USA: ACGIH®, Inc, 1999.

ACG03a American Conference of Governmental Industrial Hygienists (ACGIH). Guide to occupational exposure values - 2003. Cincinnati OH, USA: ACGIH®, Inc, 2002: 11.

ACG03b American Conference of Governmental Industrial Hygienists (ACGIH). 2003 TLVs® and BEIs® based on the documentation of the Threshold Limit Values for chemical substances and physical agents & Biological Exposure Indices. Cincinnati OH, USA: ACGIH®, Inc, 2003: 15.

Adh88 Adhikari N, Grover IS. Genotoxic effects of some pesticides: in vivo chromosomal aberrations in bone marrow cells in rats. Environ Mol Mutagen 1988; 12: 235-42.

Alb93 Albertini S, Brunner M, Wurgler FE. Analysis of the six additional chemicals for in vitro assays of the European Economic Communities' EEC aneuploidy programme using Saccharomyces cerevisiae D61.M and the in vitro porcine brain tubulin assembly assay. Environ Mol Mutagen 1993; 21: 180-92.

Ama79 Amacher DE, Paillet S, Ray VA. Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells: application to genetic toxicological testing. Mutat Res 1979; 64: 391-406.

Arb02 Arbejdstilsynet. Grænseværdier for stoffer og materialer. Copenhagen, Denmark: Arbejdstilsynet, 2002: 17 (At-vejledning C.0.1).

Ath85 Athwal RS, Sandhu SS. Use of a human X mouse hybrid cell line to detect aneuploidy induced by environmental chemicals. Mutat Res 1985; 149: 73-81.

Bar83 Barnes TB, Verlangieri AJ, Wilson MC. Reproductive toxicity of methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate (benomyl) in male Wistar rats. Toxicology 1983; 28: 103-15.

Bar93 Barale R, Scapoli C, Meli C, et al. Cytogenetic effects of benzimidazoles in mouse bone marrow. Mutat Res 1993; 300: 15-28.

Bel79 Belasco IJ. 2-$^{14}$C-Benomyl (50WP) adsorption through rat skin. Part II: Effect of time and dose applied. Wilmington DE, USA: DuPont de Nemours & Co, Biochemical Department, Research Division, Experimental Station, 1979; unpublished report, cited in FAO96, Sme01, and WHO93a.

Ben92 Bentley KS. Classification of DPX-T1991-529 (benomyl)-induced micronuclei in mouse bone marrow erythrocytes using immunofluorescence antikinetochore antibodies. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1992; unpublished report, cited in FAO96 and WHO93a.

Ben00 Bentley KS, Kirkland D, Murphy M, et al. Evaluation of thresholds for benomyl- and carbendazim-induced aneuploidy in cutured human lymhocytes using fluorescence in situ hybridization. Mutat Res 2000; 464: 41-51.

Bia97 Bianchi-Santamaria A, Gobbi M, Cembran M, et al. Human lymphocyte micronucleus genotoxicity test with mixtures of phytochemicals in environmental concentrations. Mutat Res 1997; 388: 27-32.

Bro87 Brock WJ. Acute dermal toxicity study with Benlate 50 DF fungicide in rabbits. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1987; unpublished report, cited in FAO96 and WHO93a.

Bur93 von Burg R. Benomyl. J Appl Toxicol 1993; 13: 377-81.

Bus68a Busey WM. Acute dermal $LD_{50}$ test and dermal irritation test on rabbits using wettable powder formulation (50% benomyl) with histological addendum. Falls Church VA, USA: Hazleton Laboratories, Inc, 1968; unpublished report, cited in FAO96 and WHO93a.

Bus68b Busey WM. Acute inhalation exposure test in rats using a wettable powder formulation (50% benomyl). . Falls Church VA, USA: Hazleton Laboratories, Inc, 1968; unpublished report, cited in FAO96 and WHO93a.

Car78 Carere A, Ortali VA, Cardamone G, et al. Microbial mutagenicity studies of pesticides in vitro. Mutat Res 1978; 57: 277-86.

Car82 Carter SD, Laskey JW. Effect of benomyl on reproduction in the male rat. Toxicol Lett 1982; 11: 87-94.

Dav77 Davidse LC, Flach W. Differential binding of methyl benzimidazol-2-yl carbamate to fungal tubulin as a mechanism of resistance to this antimitotic agent in mutant strains of Aspergillus nidulans. J Cell Biol 1977;72: 174-93.

DeB80 De Bertoldi M, Griselli M. Differnet test systems in Aspergillus nidulans for the evaluation of mitotic gene conversion, crossing-over and nondisjunction. Mutat Res 1980; 74: 303-24.

Des90 Desi I, Nehez M, Palotas M, et al. Experience of health status surveillance of pesticide workers in Hungary. Med Lav 1990; 81: 517-23.

DFG03 Deutsche Forschungsgemeinschaft (DFG): Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. List of MAK and BAT values 2003. Maximum concentrations and Biological Tolerance Values at the workplace Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA, 2003; rep no 39.

Dol92 Dolara PM, Savadori T, Capobianco T, et al. Sister-chromatid exchange in human lymphocytes induced by dimethoate, omethoate, deltamethrin, benomyl and their mixture. Mutat Res 1992; 283: 113-8.

Don81 Donovan SD, Krahn DF. Mutagenic evaluation in Salmonella typhimurium. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1981; unpublished report, cited in FAO96 and WHO93a.

Eas89 Eastmond DA, Tucker JD. Kinetochore localisation in micronucleated cytokinesis-blocked Chinese hamster ovary cells: A new and rapid assay for identifying aneuploidy-inducing agents. Mutat Res 1989; 224: 517-25.

EC04 European Commission: Directorate General of Employment and Social Affairs. Occupational exposure limits (OELs). http://europe.eu.int/comm/employment_social/h&s/areas/oels_en.htm.

Edw91 Edwards R, Ferry DG, Temple WA. Benomyl. In: Hayes WJ Jr, Laws ER Jr, eds. Fungicides and related compounds. New York, USA: Academic Press, 1991: 1452-4 (Handbook of pesticide toxicology; Vol 3).

Ell87 Ellis WG, Semple J, Hoogenboom E, et al. Benomyl-induced craniocerebral anomalies in fetuses of adequately nourished and protein-derived rats. Teratogen Carcinogen Mutagen 1987; 7: 357-75.

Ell88 Ellis WG, De Roos F, Kavlock RJ, et al. Relationship of periventricular overgrowth to hydrocephalus in brains of fetal rats exposed to benomyl. Teratogen Carcinogen Mutagen 1988; 8: 377-91.

Eva80 Evans EL, Mitchell AD. An evaluation of the effect of benomyl on sister chromatid exchange frequencies in cultured Chinese hamster ovary cells. Menlo Park CA, USA: SRI International, 1980; unpublished report, cited in FAO96 and WHO93a.

Eve79 Everhart LP. Benlate dust exposure survey. Wilmington DE, USA: DuPont de Nemours & Co, Biochemical Department, 1979; unpublished report, cited in WHO93a.

Eve82 Everhart LP, Holt RF. Potential benlate fungicide exposure during mixer/loader operations, crop harvest and home use. J Agric Food Chem 1982; 30: 222-7.

FAO96 Food and Agricultural Organization/World Health Organization (FAO/WHO): Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (JMPR). Benomyl. In: Pesticides residues in food – 1995 evaluations. Part II. Toxicology and environmental. Geneva, Switzerland: WHO, 1996; WHO/PCS/96.48; http://www.inchem.org/documents/jmpr/jmpmono/v95pr02.htm.

Fis78 Fiscor G, Bordas S, Stewart SJ. Mutagenicity testing of benomyl, methyl-2-benzimidazole carbamate, streptozotocin, and N-methyl-N'-nitrosguanine in S. typhimurium in vitro and in rodent host-mediated assays. Mutat Res 1978; 51: 151-64.

Fit80 Fitzpatrick K. Chinese hamster ovary cell assay for mutagenicity. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1980; unpublished report, cited in FAO96 and WHO93a.

Fos93 Foss JA. Acute neurotoxicity study of DPX-T1991-529 (Benomyl) administered orally by gavage to Crl:CD BR VAF /Plus rats. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1993; unpublished report, cited in FAO96.

Fos94 Foss JA. Subchronic neurotoxicity study of DPX-T1991-529 (Benomyl) administered orally via the diet to Crl:CD BR VAF/Plus rats. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1994; unpublished report, cited in FAO96.

Fra72 Frank KM. Eye irritation test in rabbits using a wettable powder formulation (50% benomyl). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1972; unpublished report, cited in FAO96 and WHO93a.

Fra98 Frazier LM, Hakoi K. Reproductive Hazards of the Workplace. New York, USA: Van Nostrand Reinhold, 1998: 234-7.

Fri69 Fritz SB. Acute oral ALD test in rabbits using wettable powder formulation (50% benomyl). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1969; unpublished report, cited in FAO96 and WHO93a.

Gar74 Gardiner JA, Kirkland JJ, Klopping HL, et al. Fate of benomyl in animals. J Agric Chem 1974; 22: 419-27.

Gar83a Gargus JL, Zoetis T. Eye irritation test in rabbits (Benlate PNW). Vienna VA, USA: Hazleton Laboratories, Inc, 1983; unpublished study, cited in FAO96 and WHO93a.

Gar83b Gargus JL, Zoetis T. Acute skin absorption $LD_{50}$ test on rabbits (EPA registration guidelines -Benlate PNW). Vienna VA, USA: Hazleton Laboratories, Inc, 1983; unpublished study, cited in FAO96 and WHO93a.

Gar84 Gargus JL, Zoetis T. Primary skin irritation and sensitisation test on guinea pigs (Benlate PNW). Vienna VA, USA: Hazleton Laboratories, Inc, 1984; unpublished study, cited in FAO96 and WHO93a.

Geo90 Georgieva V, Vachkova R, Tzoneva M, et al. Genotoxic activity of benomyl in different test systems. Environ Mol Mutagen 1990; 16: 32-6.

Gib95 Gibson DP, Aardema MJ, Kerckaert GA, et al. Detection of aneuploidy-inducing carcinogens in the Syrian hamster embryo (SHE) cell transformation assay. Mutat Res 1995; 343: 7-24.

Goo78 Gooch JJ. Fertility of workers potentially exposed to benomyl. Wilmington DE, USA: DuPont de Nemours & Co, 1978; unpublished report, cited in FAO96 and WHO93a.

Gou83 Goulding R. Poisoning on the farm. J Soc Occup Med 1983; 33: 60-5.

Gup75 Gupta AK, Legator MS. Chromosome aberrations in cultured human lymphocytes after treatment with fungicide 'Benlate'. In: Proceedings of the symposium on mutagenicity, carcinogenicity and teratogenicity of chemicals. New Delhi, India: Department of Atomic Energy, 1975; 95-103; cited in FAO96.

Gue81 Guengerich FP. Enzyme induction with DuPont compound H11, 202-2 and H10, 962-02. Nashville YN, USA: Vanderbilt University, School of Medicine, 1981; unpublished report, cited in FAO96 and WHO93a.

Hes91 Hess RA, Moore BJ, Forrer J, et al. The fungicide benomyl (methyl) 1-(butylcarbomyl)-2-benzimidazole carbamate causes testicular dysfunction by inducing the sloughing of germ cells and occlusion of efferent ductules. Fundam Appl Toxicol 1991; 17: 733-745

Hoo69 Hood DB. Fifteen exposure dermal tests on rabbits using a wettable powder formulation (50% benomyl) with report on spermatogenesis effects. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1969; unpublished report, cited in FAO96, Sme01, and WHO93a.

Hoo91 Hoogenboom ER, Ransdell JF, Ellis WG, et al. Effects on the fetal rat eye of maternal benomyl exposure and protein malnutrition. Curr Eye Res 1991; 10: 601-2.

Hor69 Hornberger CS. Acute dust inhalation test in rats using a wettable powder formulation (50% benomyl) with report on spermatogenesis effects. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1969; unpublished report, cited in FAO96 and WHO93a.

Hos77 Hostetler KH. Oral $LD_{50}$ test (benlate OD). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1977; unpublished report, cited in FAO96 and WHO93a.

HSE02 Health and Safety Executive (HSE). EH40/2002. Occupational Exposure Limits 2002. Sudbury (Suffolk), UK: HSE Books, 2002: 13.

Jon84 Jones DCL, Simmon VF, Mortelmans KE, et al. In vitro and in vivo mutagenicity studies of environmental chemicals. Menlo Park CA, USA: SRI International, 1984 (Report to Health Effects Research Laboratory, US Environmental Protection Agency; available from the National Technical Information Services, Springfield VA, USA; rep no: PB-84-138973).

Joo83 van Joost TH, Naafs B, van Ketel WG. Sensitization to benomyl and related pesticides. Contact Dermatitis 1983; 9: 153-4.

Kap74 Kappas A, Georgopoulos SG, Hastie AC. On the genetic activity of benzimidazole and thiophanate fungicides on diploid Aspergillus nidulans. Mutat Res 1974; 26: 17-27.

Kap76 Kappas A, Green MHL, Bridges BA, et al. Benomyl - a novel type of base analogue mutagen? Mutat Res 1976; 40: 379-82.

Kav82 Kavlock RJ, Chernoff N, Gray EL Jr, et al. Teratogenic Effects of benomyl in the Wistar rat and CD-1 mouse, with emphasis on the route of administration. Toxicol Appl Pharmacol 1982; 62: 44-54.

Küh85 Kühne G, Heise H, Plottke B, et al. Dermatitis nach Benlate-Kontakt. Z Gesamte Hyg 1985; 31: 710-1.

Lam80 Lamb MJ, Lilly LJ. An investigation of some genetic toxicological effects of the fungicide benomyl. Toxicology 1980; 17: 83-95.

Lee77 Lee KP. The two-year feeding study in rats with benomyl with supplemental pathology report. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1977; unpublished report, cited in FAO96 and WHO93a.

Lin88 Linder RE, Rehnberg GL, Strader LF, et al. Evaluation of reproductive parameters in adult male Wistar rats after subchronic exposure (gavage) to benomyl. J Toxicol Environ Health 1988; 25: 285-98.

Lis86 Lisi P, Caraffini S, Assalve D. A test series for pesticide dermatitis. Contact Dermatitis 1986; 15: 266-9.

Lit69 Littlefield NA, Busey WM. 4-hour acute inhalation exposure test in dogs using wettable powder formulation (50% benomyl). Falls Church VA, USA: Hazleton Laboratories, Inc, 1969; unpublished report, cited in FAO96 and WHO93a.

Mai92 Mailhes JB, Aardema MJ. Benomyl-induced aneuploidy in mouse oocytes. Mutagenesis 1992; 7: 303-9.

Mat81 Matsushita T, Aovama K. Cross reactions between some pesticides and the fungicide benomyl in contact allergy. Ind Health 1981; 19: 77-83.

McC83 McCooey KT, Arce GT, Sarrif AM, et al. L5178Y mouse lymphoma cell assay for mutagenicity (benomyl). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1983; unpublished report, cited in FAO96 and WHO93a.

Meb90 Mebus CA. Reproductive and fertility effects with DPX-1991-529 (benomyl). Multigeneration study in rats. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1990; unpublished report, cited in FAO96 and WHO93a.

Mon90 Monson KD. Metabolism of [phenyl (U)-$^{14}$C] carbendazim in rats. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1990; unpublished report, cited in FAO96 and WHO93a.

Mun95 Munley SM. Developmental toxicity study of DPX-T1991-529 (benomyl) in rabbits. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1995; unpublished report, cited in FAO96 and WHO93a.

NLM02 US National Library of Medicine (NLM), ed. Benomyl. In: Hazardous substances data bank (HSDB) (last revision date benomyl file: 14 January 2002; last review date: 19 September 1996); http://toxnet/nlm.nih.gov.

Pia94 Piatti E, Marabini L, Chiesara E. Increase of micronucleus frequency in cultured rat hepatocytes treated in vitro with benomyl and pirimiphos-methyl separately and in mixture. Mutat Res 1994; 324: 59-64.

Pie95 Piersma AH, Verhoef A, Dortant PM. Evaluation of the OECD 421 reproductive toxicity screening test protocol using 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl). Teratogen Carcinogen Mutagen 1995; 15: 93-100.

Pil80 Pilinskaya MA, Kurinyi AI, L'vova TS, et al. Preliminary evaluation of the cytogenetic activity and potential mutagenic hazard of 22 pesticides. Cytol Genet 1980; 14: 38-43.

Pil83 Pilinskaya MA. Investigation of the cytogenetic action of the pesticides captan and benomyl in a culture of human peripheral blood lymphocytes in the absence and presence of a system of metabolic activation. Cytol Genet 1983; 17: 29-33.

Rai89 Rainaldi G, Flori L, Colella CM, et al. Analysis by BrUdR-labelling technique of induced aneuploidy in mammalian cells in culture. Mutat Res 1989; 177: 255-60.

Ric83 Rickard LB. Mutagenicity evaluation in S. typhimurium. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1983; unpublished report, cited in FAO96 and WHO93a.

Rus78 Russell JF. Mutagenic activity of 2-benzimidazolecarbamic acid, 1-(butylcarbamoyl)-methylester in the Salmonella/ microsome assay. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1978; unpublished report, cited in FAO96 and WHO93a.

Ruz76 Ruzicska P, Peter S, Laczi J, et al. Study of the chromosome mutagenicity of Fundazol 50 WP. Egeszegtudomany 1976; 20: 74-83; cited in FAO96 and WHO93a.

Sar87 Sarver JW. Acute oral toxicity study with IN-T1991 in male and female rats. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1987; unpublished report, cited in FAO96 and WHO93a.

Sar94 Sarrif AM, Bentley KS, Fu LJ, et al. Evaluation of benomyl and carbendazim in the in vivo aneuploidy/micronucleus assay in BDF1 mouse bone marrow. Mutat Res 1994; 310: 143-9.

Sas88 Sasaki YFX. Benomyl: in vitro cytogenetics test. Tokyo, Japan: Kodaira Laboratories, Institute of Environmental Toxicology, 1988; unpublished report, cited in FAO96 and WHO93a.

Sas90 Sasaki YFX. Benomyl: micronucleus test in mice. Tokyo, Japan: Kodaira Laboratories, Institute of Environmental Toxicology, 1990; unpublished report, cited in FAO96 and WHO93a.

Sei76 Seiler JP. The mutagenicity of benzimidazole and benzimidazole derivatives. VI. Cytogenetic effects of benzimidazole derivatives in the bone marrow of the mouse and the Chinese hamster. Mutat Res 1976; 40: 339-48.

She67 Sherman H, Barnes JR, Krauss WC. Ninety-day feeding study with 1-butylcarbamoyl-2-benzimidazolecarbamic acid, methyl ester (INT-1991). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1967; unpublished report, cited in FAO96 and WHO93a.

She68 Sherman H. Three-month feeding study in dogs using wettable powder formulation (50% benomyl). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1968; unpublished report, cited in FAO96 and WHO93a.

She69a Sherman H. Acute oral LD50 test in rats using technical benomyl (>95% benomyl) and a wettable powder formulation (50% benomyl). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1969; unpublished report, cited in FAO96 and WHO93a.

She69b Sherman H. Acute oral ALD test in a dog using technical benomyl (>95% benomyl). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1969; unpublished report, cited in FAO96 and WHO93a.

She70 Sherman H. Long-term feeding study in dogs with 1-butycarbamoyl-2-benzimidazolecarbamic acid, methyl ester (INT-1991). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1970; unpublished report, cited in FAO96, Sme01, and WHO93a.

She75 Sherman H, Culik R, Jackson RA. Reproduction, teratogenic, and mutagenic studies with benomyl. Toxicol Appl Pharmacol 1975; 32: 305-15.

Shi78 Shirasu Y, Moriva M, Kato K. Mutagenicity testing on fungicide 1991 in microbial systems. Tokyo, Japan: Kodaira Laboratories, Institute of Environmental Toxicology, 1978; unpublished report, cited in FAO96 and WHO93a.

Sme01 Smegal D. Benomyl and carbendazim - Endpoint selection for incidental oral ingestion for carbendazim - 3rd report of the hazard identification assessment review committee. Washington DC, USA: US Environmental Protection Agency, Health Effects Division, 2001; HED Doc No 014506.

Sta80 Stapels RE. Teratogenicity study in the rat after administration by gavage of technical benomyl (>95% benomyl). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1980; unpublished report, cited in FAO96 and WHO93a.

Sta82 Staples RE. Teratogenicity study in the rat using technical benomyl (>95% benomyl) administered by gavage. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1982; unpublished report, cited in FAO96, Sme01, and WHO93a.

Sta90 Stahl RG Jr. In vivo evaluation of INT-1991-259 for chromosome aberrations in mouse bone marrow. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1990; unpublished report, cited in FAO96 and WHO93a.

Swe00 Swedish National Board of Occupational Safety and Health. Occupational exposure limit values and measures against air contaminants. Solna, Sweden: National Board of Occupational Safety and Health, 2000; Ordinance AFS 2000:3.

SZW03 Ministerie van Sociale Zaken en Werkgelegenheid (SZW). Nationale MAC-lijst 2003. The Hague, the Netherlands: Sdu, Servicecentrum Uitgevers, 2003: 18.

Ton81 Tong C. Hepatocyte primary culture/DNA repair assay on compound 10, 962-02 (benomyl) using mouse hepatocytes in culture. Valhalla NY, USA: Naylor Dana Institute, 1981; unpublished report, cited in FAO96 and WHO93a.

TRG00 TRGS 900. Grenzwerte in der Luft am Arbeitsplatz; Technische Regeln für Gefahrstoffe. BArbBl 2000; 2.

Tur79 Turney RT. Rat inhalation study - Benlate. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1979; unpublished report, cited in FAO96 and WHO93a.

Vic87 Vick DA, Brock WJ. Primary dermal irritation study with Benlate 50 DF fungicide in rabbits. Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1987; unpublished report, cited in FAO96 and WHO93a.

War89 Warheit DB, Kelly DP, Carakostas MC, et al. A 90-day inhalation toxicity study with benomyl in rats. Fundam Appl Toxicol 1989; 12: 333-45.

War92 Ward RS, Scott RC. Benomyl: in vitro absorption of a 500 g/kg WP formulation through human epidermis. Fernhurst, Haslemere (Surrey), UK: Imperial Chemical Industries (ICI), 1992; unpublished report, cited in FAO96 and WHO93a.

WHO93a World Health Organization/International Programme on Chemical Safety (WHO/IPCS). Benomyl. Geneva, Switzerland: WHO, 1993; Environmental Health Criteria 148.

WHO93b World Health Organization/International Programme on Chemical Safety (WHO/IPCS). Carbendazim. Geneva, Switzerland: WHO, 1993; Environmental Health Criteria 149.

Wie82 Wiechman BE. Long-term feeding study with methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate in mice (INT-1991; >95% benomyl). Newark DE, USA: Dupont de Nemours & Co, Haskell Laboratory, 1982; unpublished report, cited in FAO96 and WHO93a.

Zel90 Zelesco PA, Barbieri I, Graves JA. Use of a cell hybrid test system to demonstrate that benomyl induces aneuploidy and polyploidy. Mutat Res 1990; 242: 329-35.

## Annex

Occupational exposure limits for benomyl in various countries.

| country - organisation | occupational exposure limit | | time-weighted average | type of exposure limit | note[a] | reference[b] |
|---|---|---|---|---|---|---|
| | ppm | $mg/m^3$ | | | | |
| the Netherlands | | | | | | |
| - Ministry of Social Affairs and Employment | - | 10 | 8 h | administrative | | SZW03 |
| Germany | | | | | | |
| - AGS | - | - | | | | TRG00 |
| - DFG MAK-Kommission | - | - | | | | DFG03 |
| Great Britain | | | | | | |
| - HSE | - | 10 | 8 h | OES | | HSE02 |
| | - | 15 | 15 min | STEL | | |
| Sweden | - | - | | | | Swe00 |
| Denmark | - | 5 | 8 h | | | Arb02 |
| USA | | | | | | |
| - ACGIH | - | 10 | 8 h | TLV | A4[e] | ACG03b |
| - OSHA | - | 15[c] | 8 h | PEL | | ACG03a |
| | - | 5[d] | 8 h | STEL | | |
| - NIOSH | - | - | | | | ACG03a |
| European Union | | | | | | |
| - SCOEL | - | - | | | | EC04 |

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

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

[c] Total dust.

[d] Respirable fraction.

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