Benomyl is a broad-spectrum fungicide from the class of benzimidazoles and is widely used in great quantities. After ingestion, benomyl is transformed in the mammalian organism into the main metabolite methyl-2-benzimidazolecarbamate (carbendazim). Studies of biodistribution have shown that the substance does not accumulate in specific organs (WHO 1993). In this documentation only the germ cell mutagenic and sensitizing effects are evaluated.

**Germ cell mutagenicity**

As carbendazim is formed not only as the active main metabolite but also as the decomposition product of benomyl, the data for the genotoxicity of carbendazim already evaluated must also be taken into account in the evaluation of benomyl (“Carbendazim” 2005). The problem of contamination with aminophenazone that occurs during the production of carbendazim does not arise with benomyl.

**Genotoxicity**

**In vitro**

**Bacteria**

In the numerous bacterial gene mutation tests published, benomyl was not found to be mutagenic (Carere et al. 1978; Ficsor et al. 1978; Georgieva et al. 1990; McCarroll et al. 2002; Moriya et al. 1983). In a comprehensive study, the mutagenic effects of both ultra-pure benomyl (analytical grade) and a powder formulation with 50% benomyl were investigated in plate incorporation tests with the Salmonella strains TA98, TA1537, TA100 and TA1535. In both cases tests included the toxic dose range (benomyl: 500 mg/plate, powder formulation: 1200 mg/plate). An in-
crease in the number of revertants was not observed in any of the test strains in either the presence or the absence of metabolic activation (Sarrif et al. 1994 a).

A review published by the US Environmental Protection Agency (US EPA) about the mutagenicity tests of various chemical companies, submitted as part of the approval process for pesticides and evaluated by the US EPA, reports that mutagenic effects were observed in one of three mutagenicity tests with Salmonella typhimurium in two frameshift strains after metabolic activation and doses of 1850 mg/plate and above (McCarroll et al. 2002). Also in earlier investigations, positive results were obtained only sporadically and after high doses in bacterial mutagenicity tests. Positive results were obtained in the no longer used Salmonella strain TA1530 without metabolic activation (Seiler 1972) and increased numbers of revertants were reported in TA1535 and Escherichia coli WP2 uvrA (Kappas et al. 1976). In both cases the findings were not obtained in other studies and resulted from experiments that do not conform with present-day standards. In these reports there are no details regarding the purity of the substance or possible contamination. In the National Toxicology Program of the U. S. Department of Health and Human Services, benomyl was not found to be mutagenic in any of the mutagenicity tests carried out with frameshift and base substitution-specific Salmonella strains (TA97, TA98, TA1537 and TA100, TA1535, respectively) either in the absence or presence of metabolic activation (Zeiger et al. 1988).

In two host-mediated assays carried out after three subcutaneous applications of 500 mg/kg body weight in mice and oral doses of 4000 mg/kg body weight in rats in the form of a preparation containing 50% benomyl, no mutagenic effects could be detected in the Salmonella strains used (Ficsor et al. 1978).

It is apparent from all the investigations with bacteria that benomyl does not induce gene mutation.

Yeasts and fungi

Benomyl yielded inconsistent results as regards its ability to induce gene mutation in Saccharomyces cerevisiae and its recombinogenic effects in Saccharomyces and Aspergillus nidulans. While in two studies (Gunasekaran and Tauro 1982; Kappas and Bridges 1981) mutagenic and recombinogenic effects were described after doses of 0.25 and 10 mg/ml and above, respectively, in four other studies no increases in the frequency of mutants or recombinants were observed even after doses of up to 2.8 and 500 mg/ml (Bianchi et al. 1994; Bignami et al. 1977; de Bertoldi et al. 1980; Siebert et al. 1970). There are no details regarding contamination in any of the studies.

On the other hand, the induction of aneuploidy could be determined in Saccharomyces and Aspergillus in all the available studies with benomyl (Albertini 1991; Bianchi et al. 1994; Bignami et al. 1977; de Bertoldi et al. 1980; Kappas and Bridges 1981; Morpurgo et al. 1979).
Mammalian cells

Indicator tests
The ability of benomyl to induce sister chromatid exchange (SCE) was investigated in human lymphocytes in vitro without a metabolic activation system. While in one study a weak positive effect was observed at concentrations of up to 6 mg/ml without the addition of a metabolic activation system (which, however, was not statistically significant) (Dolara et al. 1992), another study yielded positive results in the concentration range from 0.5 to 2.0 mg/ml (Georgieva et al. 1990). The batch of benomyl was from DuPont (Athens, purity 80%). In investigations with CHO cells (a cell line derived from Chinese hamster ovary), increased SCE frequencies were observed with benomyl concentrations of 3 mM and above both in the presence and absence of a metabolic activation system; this indicates the potential ability of the substance to induce recombinogenic processes (McCarroll et al. 2002).

Primary DNA lesions, which are detected in the UDS test via the induction of DNA repair synthesis, could not be detected in two different in vitro investigations with primary rat hepatocytes (highest concentration tested: 5 mg/ml), and in mouse hepatocytes (highest concentration tested: 50 mg/ml). Data for the cytotoxicity of the tested concentrations are not available (McCarroll et al. 2002).

Chromosomal aberration tests
Investigations with mammalian cell cultures, CHO cells, human–mouse cell hybrids and human peripheral lymphocytes, in which structural chromosomal aberrations served as the genetic end point, did not yield any clear results. While a marginal increase in chromosomal aberrations was found in the hybrid cells mentioned after exposure to 1.5–15 mg/ml (Sandhu et al. 1988) and in human peripheral lymphocytes at 10 mg/ml in the absence of metabolic activation (Pilinskaya 1983), this could not be confirmed in the same study in the presence of activation with microsomes (Pilinskaya 1983). On the other hand, positive findings were reported in a chromosomal aberration test in the presence and absence of metabolic activation after concentrations of 12 mg/ml and above in a cell line of the Chinese hamster (McCarroll et al. 2002).

After the treatment of mammalian cells (Rainaldi et al. 1987; Sandhu et al. 1988; Zelesco et al. 1990) and human lymphocytes with benomyl (Raimondi et al. 1989), however, the concentration-dependent induction of aneuploidy and polyploidy was clearly demonstrated in several in vitro investigations.

In the concentration range of 0.025–3.3 mg/ml, chromosomal non-disjunction was investigated in cultured human lymphocytes in cytokinesis-blocked cells by means of fluorescence-in-situ-hybridization (FISH) using six chromosome-specific DNA samples treated with benomyl for 48 hours. The data revealed a pronounced non-linear concentration-effect curve; the concentration at which the induction of non-disjunction becomes statistically significant was given as 1.1 to 1.2 mg/ml. The simultaneous evaluation of chromosome losses and micronuclei containing...
centromeres in these binuclear lymphocytes yielded slightly higher concentrations of around 1.3–1.5 mg/ml and 1.2–1.4 mg/ml (Bentley et al. 2000).

The cytogenetic in vitro investigations in mammalian cells showed that benomyl does not have clastogenic effects, but does have aneugenic effects.

**Micronucleus tests**

In micronucleus tests, a concentration-dependent increase in cells containing micronuclei after treatment with benomyl was likewise found in mammalian cells - as was expected on the basis of the data from the chromosomal aberration studies (McCarroll et al. 2002; Piatti et al. 1994). To clarify whether the formation of micronuclei is caused by numerical or structural chromosomal aberrations, the immunocytochemical technique of CREST staining was used, with which centromere material in the micronuclei can be made visible. Thus, after the in vitro treatment of mouse L cells with 10, 15 or 20 mg/ml (Sternes and Vig 1989) and of cytokinesis-blocked CHO cells with 3.44, 10.3 or 34.4 mg/ml (Eastmond and Tucker 1989), a statistically significant increase in benomyl-induced micronuclei was determined at concentrations of 10 mg/ml and above; around 69% (Sternes and Vig 1989) and 75% were CREST-positive (Eastmond and Tucker 1989), and thus the result of chromosomal non-disjunction. The proportion of kinetochore-negative micronuclei at 10 mg/ml was about 12% and 19%.

The results of in vitro micronucleus tests confirm the aneugenic effects of benomyl.

**Gene mutation tests**

In an HPRT gene mutation test with cultured cells from the Chinese hamster, benomyl yielded negative results into the cytotoxic concentration range (McCarroll et al. 2002). A mouse lymphoma TK+/- assay with carbendazim (which detects gene or chromosomal mutations and yielded positive results), contained the comment that additional studies had shown that not carbendazim itself, but contamination with aminophenazone had caused these mutagenic effects (McCarroll et al. 2002).

The results of the mutation tests with mammalian cells confirm the findings with bacteria, which show that benomyl and its main metabolite carbendazim do not induce gene mutations.

**In vivo**

**Drosophila**

In a test for X-chromosomal recessive lethal mutations in Drosophila (SLRL test), the administration of benomyl concentrations of 1000 mg/ml with the diet did not lead to an increase in the frequency of lethal gene or chromosome mutations (Lamb and Lilly 1980).
Mammals

Somatic cells

Micronucleus tests
In a micronucleus test with mouse bone marrow cells, a significant increase in the incidence of micronuclei was detected 48 hours after the administration of single oral benomyl doses of 2500 and 5000 mg/kg body weight (corresponding to 8.6 and 17.2 mmol/kg), but not after 100 mg/kg body weight (0.3 mmol/kg). Using fluorescence-labelled, kinetochore-specific antibodies, it could be demonstrated that 82% of the micronuclei occurred as a result of chromosome losses (Sarrif et al. 1994 b). Both doses led also to a decrease in the PCE:NCE ratio, which indicates the bioavailability of the substance and moreover toxic effects in the bone marrow. In an earlier study, positive results were obtained in a micronucleus test with the mouse after doses of 1000 mg/kg body weight (Seiler 1976). The results of a micronucleus test with mice were positive also 36 hours after the administration of doses of 1000 mg/kg body weight (Barale et al. 1993). Toxic effects on the bone marrow were not detected at this dose. The results of two other micronucleus tests (which were available to the US EPA as an unpublished report), in which mice were given oral doses of benomyl, were regarded as positive after doses of 1000 and 2500 mg/kg body weight; another test with the mouse did not lead to an increase in cells containing micronuclei up to the highest dose tested of 5000 mg/kg body weight (McCarroll et al. 2002).

In rats, the long-term oral administration of doses of 10 to 200 mg/kg over a period of 70 days led merely to a weak increase in the frequency of bone marrow cells containing micronuclei (Georgieva et al. 1990). In this study a commercial preparation containing 80% benomyl was used.

Chromosomal aberration tests
The determination of structural chromosomal aberrations in the bone marrow of the mouse yielded contradictory results. While the results in one report, in which 1250 mg/kg body weight was administered orally, were regarded by the US EPA as positive (McCarroll et al. 2002), the oral administration of up to 5000 mg/kg body weight to B6D2F2/Cr-1Br mice did not lead to an increase in structurally aberrant bone marrow cells (WHO 1993). Single doses of 1000 mg/kg body weight likewise did not lead to an increase in the number of structural aberrations in the bone marrow of the mouse (Barale et al. 1993). In an in vivo study with groups of 5 rats, benomyl doses of 0, 250, 500 or 1000 mg/kg body weight were administered twice intraperitoneally at an interval of 24 hours. The bone marrow was prepared six hours after administration of the second dose. At the high dose, the number of chromosomal breaks and chromatid breaks was significantly increased in 200 metaphases (Adhikari and Grover 1988).
Gene mutation tests
There are no investigations available of the induction of gene mutations in mammals in vivo.

Male germ cells

It is known that benomyl has adverse effects on the male reproductive organs of various mammals (WHO 1993). Not only the somatic Sertoli cells of the reproductive organs were affected, but also the germ cells themselves (Hess and Nakai 2000; Nakai and Hess 1994; WHO 1993). The benomyl metabolite carbendazim and not benomyl itself is responsible for the toxic effects on the testes in rats (Lim and Miller 1997).

In dominant lethal tests with male CD rats given up to 0.25% benomyl with their diet for seven days (corresponding to a maximum dose of 250 mg/kg body weight), benomyl did not lead to an increase in dominant lethal mutations after mating with untreated female animals (Sherman et al. 1975). Likewise, no effects which indicate the induction of dominant lethal mutations were observed after long-term oral administration to male rats of a preparation containing 80% benomyl in doses of 10 or 50 mg/kg body weight and day over a period of 70 days and subsequent mating for three weeks with untreated females (Georgieva et al. 1990).

Female germ cells

The oral administration of a benomyl suspension in olive oil in doses of 0, 500, 1000, 1500, 1750 or 2000 mg/kg body weight to female ICR mice during the pre-ovulatory maturation of the oocytes, that is immediately after the gonadotropin-mediated stimulation of ovulation, led to a statistically significant, dose-dependent increase in the frequency of aneuploid metaphase II oocytes, which were isolated 17 hours after the administration of gonadotropin (Mailhes and Aardema 1992). This shows that in high doses the substance induces chromosomal non-disjunction in female germ cells in the first meiotic division. In addition, in this study diploid oocytes occurred in the treated animals, which were not observed in the control animals. The absence of structural chromosomal aberrations is plausible in view of the mechanism of action of benomyl, as S phase-dependent clastogens cannot be detected with the study design used.
### Allergenic effects

#### Effects in humans

#### Sensitizing effects on the skin

It is reported in a review that benomyl caused skin problems in 35 agricultural workers in California between 1982 and 1989 (O’Malley et al. 1995). There are no details regarding the type of skin problems or data from patch test findings.

In addition, there are several reports of contact sensitization in agricultural and horticultural workers occupationally exposed to benomyl (see Table 1).

#### Table 1  Reports of patch test reactions to benomyl in occupationally exposed patients with contact dermatitis or suspected contact allergy

<table>
<thead>
<tr>
<th>Persons tested</th>
<th>Concentration (vehicle)</th>
<th>Results</th>
<th>Contact/Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>a rose cultivator with a 2-year history of occupation-related skin disorders on the hands and face</td>
<td>0.1% (water)</td>
<td>positive (no other details)</td>
<td>skin reactions in the summer after using benomyl, which had already been in use for 5 years; in addition reactions to 2,2-dichlorovinyl dimethyl phosphate also used and to “lanolin alcohol”</td>
<td>Fregert 1973</td>
</tr>
<tr>
<td>2 plant growers and a florist with dermatitis on the hands</td>
<td>1% (petrolatum)</td>
<td>1× 1+, 2+ and 3+ (after 48 or 72 hours)</td>
<td>in 1 each of the 3 persons tested also reactions to captan (3+), dithianon (2+) or dienochlor (2+) respectively; no reactions in 10 control persons to 1% benomyl</td>
<td>van Joost et al. 1983</td>
</tr>
<tr>
<td>10 female workers employed in mushroom cultivation and 2 female gardeners</td>
<td>0.05%, tested as the finished product (water)</td>
<td>all positive (no other details)</td>
<td>benomyl concentration in the finished product 50%; the two gardeners (cucumber cultivation and treatment of bulbs) were tested also with 0.25%</td>
<td>Jung et al. 1987</td>
</tr>
<tr>
<td>1 female gardener with dermatitis on the eyelids and another with skin disorders on the face and neck</td>
<td>0.1%, tested as the finished product (water)</td>
<td>2+ and 3+ (after 72 hours)</td>
<td>tending and gathering of carnations and employment in fruit cultivation; benomyl concentration in the finished product 50%</td>
<td>Jung et al. 1989</td>
</tr>
<tr>
<td>Persons tested</td>
<td>Concentration (vehicle)</td>
<td>Results</td>
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<td>References</td>
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<td>----------------</td>
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</tr>
<tr>
<td>1 plant grower with a 2-year history of dermatitis on the hands</td>
<td>1% (petrolatum)</td>
<td>positive (no other details)</td>
<td>contact with leaves treated with benomyl; no reactions in 10 control persons</td>
<td>van Ketel 1976</td>
</tr>
<tr>
<td>7 female workers employed in mushroom cultivation with skin changes on the hands and forearms</td>
<td>0.01% and 0.05% (not specified)</td>
<td>3× 2+ reactions to 0.05%, 3× 1+ reactions to 0.05%, 1× 1+ reaction to 0.01%</td>
<td>two wettable fungicide powders with a benomyl concentration of 50%; one year after avoiding occupational contact, less pronounced reactions in 4 patients on re-testing; on renewed investigation after 7 years, patch test reactions only in 2/7 persons; no reactions in 25 control persons</td>
<td>Kühne et al. 1985</td>
</tr>
<tr>
<td>25 workers currently employed in mushroom harvesting and 3 former employees of the company, 4 former employees with occupational dermatitis and 2 employees with skin disorders from contact with benomyl</td>
<td>0.1%, tested as the finished product (petrolatum)</td>
<td>0/34 positive</td>
<td>benomyl used by the company for more than 10 years; commercial product with 50% benomyl (purity 95%) and 50% saccharose tested; in 0/18 of the persons tested likewise no reaction to 0.1% preparation in water; an initial weak patch test reaction to 0.1% benomyl in water was not reproducible in the 2 employees affected</td>
<td>Larsen et al. 1990</td>
</tr>
<tr>
<td>21 former and 32 current agricultural workers, and 52 persons not employed in agriculture</td>
<td>0.1% (petrolatum)</td>
<td>1× 2+ (after 48 and 72 hours)</td>
<td>a reaction in one person not employed in agriculture who was not known to have been exposed to benomyl</td>
<td>Lisi et al. 1986</td>
</tr>
<tr>
<td>31 former and 116 current agricultural workers and 144 patients with a non-allergic cause of skin disorders</td>
<td>0.1% (petrolatum)</td>
<td>0/291 positive</td>
<td>–</td>
<td>Lisi et al. 1987</td>
</tr>
<tr>
<td>Persons tested</td>
<td>Concentration (vehicle)</td>
<td>Results</td>
<td>Contact/Comments</td>
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<tr>
<td>126 Japanese agricultural workers, 36 with skin disorders</td>
<td>1% (petrolatum)</td>
<td>7× 1+ and 1× 2+ reactions in 42 exposed persons and 12× 1+ and 3× 2+ reactions in 84 persons not exposed</td>
<td>reactions in 7/17 women previously exposed to benomyl and in 14/72 women not previously exposed to benomyl</td>
<td>Matsushita and Aoyama 1981</td>
</tr>
<tr>
<td>37 employees from banana plantations with skin disorders and 23 employees without skin disorders</td>
<td>1% (petrolatum)</td>
<td>reactions in 4/60 (no other details)</td>
<td>reactions in 2 employees from each group</td>
<td>Penagos et al. 2004</td>
</tr>
<tr>
<td>4 female plant growers of Japanese origin with skin disorders on various exposed areas</td>
<td>10%, tested as the finished product (olive oil)</td>
<td>4× 2+ reactions (after 48 hours)</td>
<td>in 3 other female Japanese employees with skin disorders patch testing was not carried out; finished product probably contained 50% benomyl; no skin disorders in 10 exposed employees of Mexican origin; no reactions to 10% and 20% in olive oil or to the undiluted product in 3 control persons; 2+ reaction to 20% and to the undiluted product after ultraviolet irradiation of the test area in one Japanese medical assistant</td>
<td>Savitt 1972</td>
</tr>
<tr>
<td>273 patients</td>
<td>0.1% (petrolatum)</td>
<td>4/273 positive (2+ or 3 + reactions)</td>
<td>results of a multicentric investigation in 21 dermatological practices; in addition 9× questionable reactions</td>
<td>Scheuer et al. 1992</td>
</tr>
<tr>
<td>7 agricultural workers with contact dermatitis mainly on the hands or arms</td>
<td>0.1% (petrolatum)</td>
<td>2× 2+ reactions (after 48 hours)</td>
<td>7 of 14 agricultural workers with skin disorders tested</td>
<td>Schuman and Dobson 1985</td>
</tr>
</tbody>
</table>
In patch tests, a variety of preparations were used; in some cases the patch test included pesticide-products containing benomyl. As benomyl concentrations of as little as 1% or 2% can lead to irritant effects in the patch test (Jung et al. 1987; Kühne et al. 1985), a test concentration of 0.05% to 0.1% in water was recommended to avoid irritant reactions (Jung 1986; Jung et al. 1987). Sensitization was observed more frequently after contact with plants and fungi treated with benomyl and less frequently after spraying benomyl (Fregert 1973; van Joost et al. 1983; van Ketel 1976; Kühne et al. 1985). Several studies report that Japanese workers and workers of Japanese origin react more frequently to benomyl (Matsushita and Aoyama 1981; Savitt 1972; Ueda et al. 1994), but other studies show that sensitization occurs also in other exposed employees (Jung et al. 1987; Kühne et al. 1985). In three studies, patch tests did not yield any evidence of sensitization after exposure to benomyl (Larsen et al. 1990; Lisi et al. 1986, 1987). The authors of the two investigations in Italy point out that benomyl was not widely used there at the time of the investigation (Lisi et al. 1987). Compared with the widespread use of benomyl overall, the number of sensitized persons is small. A possible reason for this may be the fact that benomyl is rarely used continuously, but often alternately with numerous different types of pesticide. It is therefore difficult to assign the source of sensitization and the only temporary skin problems seldom give cause for a medical investigation (Fregert 1973; van Joost et al. 1983). If contact with the substance is avoided for an adequate period, retesting several years later might not detect slight sensitization (Kühne et al. 1985).

In addition, sensitization to structurally related pesticides is thought to be responsible for cross-reactions with benomyl (Larsen et al. 1990). An investigation in 126 agricultural workers in Japan who treated plant crops with benomyl took into account possible cross-reactions (Table 1). Reactions to benomyl were found in this

Table 1 (Continued)

<table>
<thead>
<tr>
<th>Persons tested</th>
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<th>Contact/Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>107 employees from chrysanthemum cultivation</td>
<td>1% (petrolatum)</td>
<td>9/107 positive (at least 1+, after 48 hours)</td>
<td>96% of the persons tested were potentially occupationally exposed to benomyl; occupation-related symptoms in 6/9 patients; reactions in 5/59 men and 4/48 women; testing carried out shortly after cutting the flowers in spring; no reactions in control persons not employed in agriculture (no other details)</td>
<td>Ueda et al. 1994</td>
</tr>
</tbody>
</table>
investigation also in employees not previously exposed, and, in particular, in workers who sprayed other pesticides, especially diazinon, thiobencarb, chlorthalonil and copper(II)oxychloride (Matsushita and Aoyama 1981). These findings cannot be interpreted as the expression of immunological cross-reactivity with benomyl, however, taking the structural divergence into account and as co-exposure and unspecific reactions cannot be excluded.

**Sensitizing effects on the airways**

There are no data available for the sensitizing effects of benomyl on the airways.

**Results of Animal Studies**

**Sensitizing effects on the skin**

In a maximization test in 10 female Hartley guinea pigs with intradermal and topical induction with 5% or 25% benomyl in water, all 10 animals reacted 24 and 48 hours after epicutaneous provocation with concentrations of 2% or 0.5% benomyl in water. In further investigations there were no cross-reactions with benomyl after pretreatment of the guinea pigs with thiophanate-methyl or n-butylcarbamate. Of the animals pretreated with methyl-2-benzimidazole carbamate (carbendazim) for induction, two and three animals, respectively, reacted to 0.5% and 2% benomyl on provocation (Matsushita et al. 1977).

Another maximization test in guinea pigs with intradermal and topical induction with 5% or 25% benomyl in water led to reactions in all ten animals 24 and 48 hours after provocation with topical concentrations of 2% and 0.2% benomyl in water. The pure substance was not used, but a finished product probably containing 50% benomyl. Reactions to 2% benomyl, regarded by the authors to be cross-reactions, but not to 0.2% benomyl, were found also in 20% to 30% of the animals pretreated with diazinon, iprobenfos, chlorthalonil and copper(II)oxychloride (Matsushita and Aoyama 1981). As the substances are not closely related structurally, it is, however, questionable whether these are really immunological cross-reactions with benomyl. There are no data available for control tests in control animals pretreated with Freund’s complete adjuvant; unspecific reactions in the sense of an “angry back” phenomenon cannot, therefore, be excluded.

In a test with 10 Hartley guinea pigs, a 0.1% solution of a finished product containing 50% benomyl in physiological saline was injected into the animals once a week for four weeks for induction. The 10 control animals were given injections of physiological saline only. Two weeks after the last injection, 8% or 80% of a finished product containing benomyl in physiological saline was applied epicutaneously to the dorsal skin of the animals; the control animals were treated with physiological saline only. Of 10 animals, 2 reacted to the 8% preparation and 7 animals reacted to
the 80% preparation. The control animals did not react to either the induction
treatment or the provocation (WHO 1993). Guinea pigs treated with technical-
grade benomyl or a 50% formulation with saccharose either intradermally or with
repeated applications to the abraded skin for induction, produced slight to moder-
ate erythema after provocation (WHO 1993).

Sensitizing effects on the airways

There are no data available of the sensitizing effects on the airways of benomyl.

Manifesto

Benomyl was not found to be mutagenic in bacterial reversion tests. Evidence of
mutagenic effects in mammalian gene mutation tests with mouse lymphoma cells is
relativized by the possibility of mutagenic impurities; also chromosome losses could
be responsible for the positive results. The view that mutagenic effects are not the
cause of gene mutations is in agreement with the overall conclusion of the prokary-
otic and eukaryotic genotoxicity studies carried out with the active benomyl meta-
bolite carbendazim ("Carbendazim" 2005). Benomyl is therefore not expected to be
incorporated in the DNA or to lead to gene mutations as a result of structural simi-
larities with base analogues. The absence of a gene mutation-inducing effect corre-
sponds with the negative findings in the in vitro tests for DNA damage.

Benomyl was found to be weakly clastogenic merely in one in vivo chromosomal
aberration study with rat bone marrow cells. Chromosome breaking potential could
not be found in any other in vivo or in vitro studies. Also this corresponds with the
results for carbendazim.

There are clearly positive findings for the induction of micronuclei in various in
vitro and in vivo systems. Benomyl led in each case to a dose-dependent increase in
the frequency of micronuclei.

By means of chromosomal and centromere-specific staining and hybridization
techniques, chromosomal non-disjunction could be identified unequivocally as the
cause of micronuclei in the somatic cells; the same was found also in the corre-
sponding carbendazim studies. This mechanism of action is plausible as benomyl
and the active metabolite carbendazim are known to interact with microtubular
proteins, and to bind in particular with the β subunit of tubulin and thus inhibit the
polymerization of the microtubuli necessary for chromosome segregation (Davidse
and Flach 1977). While binding to the tubulin subunits in the cell probably takes
place in a concentration-dependent manner, it can be presumed that the impair-
ment of the spindle function and chromosome distribution, in other words the bio-
logical effect itself, does not occur until concentrations which prevent the polymer-
ization of a sufficient number of tubulin structures and thus the attachment of a
chromosome (Bentley et al. 2000).
Such a mode of action would lead to a concentration below which the effect of the substance in the cell would not lead to a biological effect. The in vitro data of Bentley et al. (2000), which show there to be a pronounced non-linear concentration–effect relationship, yielded a theoretical LOAEL of 1.1 mg/ml for the induction of aneuploidy by benomyl.

Although benomyl was not found to have mutagenic effects in male germ cells, it could be shown that the substance induces aneuploidy in the female germ cells of mice as a result of its particular mechanism of action. Therefore, and in analogy to the classification of carbendazim, benomyl has been classified in Category 3A for germ cell mutagens. If a Category 4 for substances with aneugenic effects should be established for germ cell mutagens, it must be re-evaluated whether benomyl can be classified in this category.

Several case reports and positive results in studies with occupationally exposed persons indicate that benomyl has contact sensitizing properties, although the number of proven cases of sensitization is small compared with the widespread use of the substance. Positive results in several studies with animals, both with and without the use of an adjuvant, likewise provide evidence of contact sensitizing effects of benomyl. Benomyl is therefore designated with an “Sh”. There are no data available for sensitizing effects on the airways; benomyl is therefore not designated with an “Sa”.

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