cholesterol, sodium and potassium as well as haematological parameters. Plasma protein electrophoresis was performed and glucose and lipoprotein levels were determined. No signs of toxicity were noted, except that the monkeys receiving 5 mg/kg bw/day of patulin started to reject their food during the last 3 days of the experiment. No statistically significant differences were observed in any of the parameters studied (Garza *et al.*, 1977).

### *2.2.3 Long-term toxicity/carcinogenicity studies*

#### *2.2.3.1 Rats*

Subcutaneous injections of 0.2 mg of patulin in 0.5 ml of arachis oil were administered biweekly for 61 or 64 weeks to 2 groups of 5 male Wistar rats, weighing 100 g at the start of the experiment. Local (fibro)sarcomas was produced at the injection site in 4/4 and 2/4 rats surviving at the time when the first tumour was observed. No metastases were observed, and of 3 tumours tested only one was transplantable in 3 of 12 recipient rats. Control animals receiving arachis oil did not develop local tumours (Dickens & Jones, 1961).

Patulin in water containing 0.05% lactic acid was administered by gavage twice weekly to 50 female SPF Sprague-Dawley rats at a dose of 1 mg/kg bw for 4 weeks, and 2.5 mg/kg bw for the following 70 weeks (total dose: 358 mg/kg bw of patulin). No effects were observed on weight gain or survival. No significant differences were observed in tumour incidence. The occurrence of 4 forestomach papillomas and 2 glandular stomach adenomas, as compared to none in the control animals, is noteworthy. The Committee noted a discrepancy between the reported duration of the study (64 weeks) and the reported duration of administration (74 weeks) (Osswald *et al.*, 1978).

Groups of 70 FDRL Wistar rats of each sex were exposed to 0, 0.1, 0.5, or 1.5 mg/kg bw/day of patulin in citrate buffer by gavage 3 times/week for 24 months. The rats were derived from the $F_1$ generation of a 1-generation reproductive toxicity study. Mortality was increased in both sexes at the highest dose: all males had died by 19 months; 19% of females survived until termination at 24 months. Body weights of males were reduced at the mid and high dose, but for females body weights were comparable in all groups. No difference in tumour incidence was observed. The NOEL in this study was 0.1 mg/kg bw, administered 3 times weekly (Becci *et al.*, 1981).

### *2.2.4 Reproductive toxicity studies*

#### *2.2.4.1 Rats*

Groups of Sprague-Dawley rats (30/sex/group) received doses of 0, 1.5, 7.5, or 15.0 mg/kg bw/day of patulin in citrate buffer by gavage 5 times per week for 7 weeks before mating. The pregnant dams were gavaged daily at the same levels during gestation. Half the dams were sacrificed on day 20 of

gestation, and used for teratological evaluation. The remaining dams were allowed to produce the $F_1$ generation. Some of the $F_0$ and $F_1$ males were used for a dominant lethal experiment. Twenty three controls and 15 low-dose animals per sex were continued to produce an $F_2$ generation. One-half of the latter generation were again used for teratological evaluation. Haematological and blood chemistry examinations were performed on 10 males and 10 females of the $F_1$ generation 23 days after weaning. The only lesion found at necropsy of parent animals was gaseous distension of the GI tract. All treated males of the $F_0$ generation had a dose-related reduction in weight gain. High mortality occurred at 7.5 and 15.0 mg/kg bw/day in both males and dams. Although litter size at 7.5 mg/kg bw/day was comparable to controls, survival of male progeny was severely impaired. At the 1.5 mg/kg bw/day level, pup growth of both sexes was reduced, and there was increased mortality among the $F_2$ females. No significant alterations were found in the haematology and blood chemistry levels in selected animals of the $F_1$ generation (Dailey *el al.*, 1977b).

Groups of FDRL Wistar rats (50/sex/group) were exposed to 0, 0.1, 0.5, or 1.5 mg/kg bw/day of patulin in citrate buffer by gavage for 4 weeks before mating, and pregnant females were dosed through gestation and lactation. The parent generation was sacrificed after weaning. Body-weight gain was comparable among groups. Ten females died in the high-dose group. Reproductive parameters such as mating success, litter size, fertility, gestation, viability, and lactation indices, and pup weight at birth, at 4 days and at weaning, were not statistically different among experimental groups. Histopathological evaluation of grossly abnormal tissues of the $F_0$ generation did not show any effects of patulin treatment. The $F_1$ generation was used for a 2-year toxicity/carcinogenicity study (see section 2.2.3.1) (Becci *et al.*, 1981).

### *2.2.5 Special studies on antibiotic activity*

Twelve species of bacteria and two species of yeast were tested for sensitivity against 11 different mycotoxins, including patulin, using a disc diffusion assay. *Bacillus brevis* appeared to be the most sensitive microorganism. The lowest amount that could be detected under optimal conditions was 1 μg/disc for patulin (Madhyastha *et al.*, 1994).

Clear synergy was shown with patulin plus rifampin and patulin plus bottromycin. Synergy of patulin with efrotomycin was weak and there was no synergy of patulin plus kasugamycin (Dulaney & Jacobsen, 1987).

### *2.2.6 Special studies on antitumour activity*

A comparison was made between the cytotoxicity and antitumour activity of patulin and five structural analogs (isopatulin, dehydroisopatulin, dimethylisopatulin, trimethylisopatulin and isopropylisopatulin). *In vitro* assays using L1210 and P 388 cells showed that the structure of the pyranic ring as

well as the nature of the substituents influenced the observed activities. Among the five structural analogs of patulin assayed *in vivo* against Ehrlich carcinoma, L1210 and P388 leukemias, dehydroisopatulin was the only one being active on all 3 types of tumours at a dose of 100 mg/kg bw/day. The ratios between the $LD_{50}$ in mice and the active dose was 5, while with patulin it was 10 (Seigle-Murandi *et al.*, 1992).

### *2.2.7 Special studies on cytotoxicity*

The $ID_{50}$ (inhibitory dose) of patulin tested on the protozoan *Tetrahymena pyriformis* was 0.32 μg/ml (Nishie, *et al.*, 1989).

Patulin at a concentration of 3.2 μg/ml inhibited by 50% the growth rate of the ciliate *Tetrahymena* (Bürger *et al.*, 1988).

To evaluate its inhibitory effect on cells, hepatoma tissue culture cells in suspension were incubated in the presence of 30 μM of patulin for 7 h and investigated by transmission and scanning electron microscopy. The most significant difference observed between treated and control cells was the disorganization of the cytoplasmic microfilaments in the treated cells (Rihn, *et al.*, 1986).

In an immortalized rat granulosa cell line, effects of patulin on GSH levels and alterations in the partitioning of rhodamine 123 were detected at 0.1 μM within 1 h. Alterations in $Ca^{2+}$ homeostasis, intracellular pH and gap junction mediated intercellular communication were detected between 1 and 2 h with 1.0 μM patulin (Burghardt *et al.*, 1992).

A study in cultural renal cells on the effect of patulin on ion influx, and the influence of dithiothreitol and glutathione on patulin effects was performed. It was hypothesized that patulin altered intracellular ion content via $Na^+$-$K^+$ ATPase and non-$Na^+$-$K^+$ ATPase mechanism (Hinton *et al.*, 1989).

In cultured renal cells LLC-$PK_1$, concentrations of patulin above 10 μM caused a transient increase in intracellular electronegativity (< 1 h) followed by a sustained depolarization (> 1 h), which was correlated with complete $Na^+$ influx, $K^+$ efflux, total LDH release, and bleb formation. However, patulin concentrations of 5-10 μM caused a sustained increased intracellular electronegativity (4-8 h) which was associated with partial $Na^+$ influx and $K^+$ efflux, no significant LDH release, and relatively few blebs. The hyperpolarizing effect may be a result of increased intracellular electronegativity. The toxic effects of patulin are irreversible in LLC-$PK_1$ cells, even after short pretreatment with patulin (Riley *et al.*, 1990).

In LLC-$PK_1$ cells exposed to 50 μM patulin lipid peroxidation, abrupt calcium influx, extensive blebbing and total LDH release appeared to be serially connected events with each representing a step in the loss of structural

integrity of the plasma membrane. Patulin also caused depletion of nonprotein sulfhydryls, increased $^{86}Rb^{+}$ efflux, dome collapse and eventually the loss of cell viability (Riley & Showker, 1991).

### *2.2.8 Special studies on genotoxicity*

The results of *in vitro* and *in vivo* genotoxicity studies with patulin are summarized in Tables 2 and 3, respectively.

Patulin was negative in mutagenicity tests with *S. typhimurium* but was clearly positive in the initiator tRNA acceptance assay for carcinogens (Hradec & Vesely, 1989).

The mutagenic effect of patulin was studied with a mutant of bacteriophage M13am6H1. A 50% decrease in liberation of M13 phage per cell ($ED_{50}$) was observed at a concentration of 0.85 μg patulin/ml, and a 50% decrease in growth rate of *E. coli* host cells was observed at a much higher concentration of the mycotoxin (6.3 μg/ml). The reversion frequency of M13am6H1 to the wild-type phenotype in the presence of patulin compared to the spontaneous reversion increased by a factor of 7-19.5 depending on the addition of patulin to bacteria or phages only or simultaneously.

The same authors studied the effects of patulin on protein and DNA synthesis. At a concentration of 3.2 μg/ml, the protein synthesis of the ciliate *Tetrahymena* was inhibited by 85% and the RNA synthesis by 86% compared with the control. Four hours after addition of patulin, DNA synthesis was reduced to 20%; it rose to the value of the control after an additional 2 h. An *in vitro* system could be developed consisting of permeabilized cells of *Tetrahymena*. This system allowed the separation of regulatory and secondary effects induced by patulin. Patulin reduced DNA synthesis by 50%, whereas RNA and protein synthesis were less inhibited than in the *in vivo* system (Burger *et al.*, 1988).

**Table 2. Results of *in vitro* genotoxicity assays on Patulin**

| Test system | Test object | Concentration | Result | Reference |
|---|---|---|---|---|
| Ames test | *E. coli* M 13am6H1 | 1 μg/ml (to phage) &/or 5 μg/ml (to bacteria) | Positive | Burger *et al.*, 1988 |
| Ames test(3,4) | *S. typhimurium* TA98, TA100 | 0.01, 0.1, 1, 10, 100, & 500 μg/plate | Negative | Ueno *et al.*, 1978 |
| Ames test(3) | *S. typhimurium* TA98, TA100, TA1535, TA1537 | 0.25 2.5, 25 & 250 μg/plate | Negative | Wehner *et al.*, 1978 |
| Ames test(3) | *S. typhimurium* TA1535, TA1537, TA1538 | 0.1, 1, 10 & 100 μg/plate | Negative | Kuczek *et al.*, 1978 |

**Table 2 (cont'd)**

| Test system | Test object | Concentration | Result | Reference |
|---|---|---|---|---|
| Ames test(5) | *S. typhimurium* TA98, TA100 | 5, 10, 20 & 30 μg/plate | Negative | Von Wright & Lindroth, 1978 |
| Ames test(3,5) | *S. typhimurium* TA100, TA1538 | <0.0065 μmoles/plate | Negative | Bartsch *et al.*, 1980 |
| Ames test(5) | *S. typhimurium* TA102 | 12-960 μg/plate Conc. >35 μg/plate were toxic | Negative | Würgler *et al.*, 1991 |
| Chromotest(3) | *E. coli* K12 PQ37 | 0.01, 0.02 & 0.05 μg/ml | Positive (No S-9) Negative (with S-9) | Auffray & Boutibonnes, 1987 |
| SOS Chromotest | *E. coli* K12 | 0.01, 0.02 & 0.05 μg/l | Weakly positive | Auffray & Boutibonnes 1988 |
| Chromotest(2) | *E. coli* PQ37 | 0.001-30 μg/ml | Negative | Krivobok *et al.*, 1987 |
| Recombinogenesis | *B. subtilis* | 20 & 100 μg/disc | Postive | Ueno & Kubota, 1976 |
| Prophage induction | *E. coli* X8011 (lambda) | 5, 10, 25 & 50 μg/ml | Positive | Lee & Roschenthaler, 1986 |
| Spot test | *E. coli* K12 | 1-10 μg/assay(2) | Positive | Auffray & Boutibonnes 1986 |
| Reverse mutagenesis(3) | *S. cerevisiae* D-3 | 50 /No S-9) & 100 (with S-9) μg/plate | Positive | Kuczuk *et al.*, 1978 |
| Forward mutagenesis | *S. cerevisiae* (haploid) | 10, 25, 50 & 75 μg/ml | Positive | Mayer & Legator 1969 |
| Forward mutagenesis (8-azoquanine resistance) | FM3A mouse mammary carcinoma cells | 0.032, 0.1, & 0.32 μg/ml | Positive | Umeda *et al.*, 1977 |
| SOS microplate assay(3) | *E. coli* PQ37 | 2, 8, 24 μg/ml | Negative | Sakai *et al.*, 1992 |
| Somatic mutations | *Drosophila melanogaster* | $3.2x10^{-2}$, $3.2x10^{-3}$, $4x10^{-4}$ M | Weakly positive | Belitsky *et al.*, 1985 |
| Chromosome aberration induction | FM3A mouse mammary carcinoma cells | 0.032, 0.1 & 0.32 μg/ml | Positive | Mori *et al.*, 1984 |
| Chromosome aberration induction(3) | Chinese hamster V79-E cells | 1, 2.5, 5 & 10 μM | Positive (No S-9) Negative | Thust *et al.*, 1982 |
| Chromosome aberration induction | Human leucocytes | 3.5 μM | Positive | Withers, 1966 |
| Cell cycle retardation | Prim. Chinese hamster cells | 0.5, 1 & 2 μg/ml | Positive | Kubiak & Kosz-Vnenchak, 1983 |
| Cell cycle retardation | Human peripheral blood lymphocytes | 0.075 & 0.30 mg/ml | Positive | Cooray *et al.*, 1982 |

Table 2 (cont'd)

| Test system | Test object | Concentration | Result | Reference |
|---|---|---|---|---|
| Cell cycle retardation | Human peripheral blood lymphocytes | 0.075 & 0.30 μg/ml | Positive | Cooray *et al.*, 1982 |
| Sister chromatid exchange induction(3) | Chinese hamster V79-E cells | 1, 2.5, 5 &10 μM | Negative | Thust *et al.*, 1982 |
| Sister chromatid exchange induction | Prim. Chinese hamster cells | 0.5, 1 & 2 mg/ml | Positive | Kubiak & Vnencha, 1983 |
| Sister chromatid exchange induction | Human peripheral blood lymphocytes | 0.075, 0.10, 0.20 & 0.30 mg/ml | Weakly positive | Cooray *et al.*, 1982 |
| DNA synthesis retardation | *T. pyriformis* | 3.2 μg/ml | Positive | Burger *et al.*, 1988 |
| DNA synthesis retardation | AWRF cells<br>CHO cells | 1, 2, 4 & 8 μg/ml<br>0.24, 0.5, 1,2,4 μg/ml | Positive | Stetina & Votova, 1986 |
| DNA breakage | ColE1 plasmid<br>Lambda DNA | 0.25, 0.5, 1.0 & 5.0 mM(1)<br>0.5, 1, 5, 10 & 14 mM | Negative | Lee & Roschenthaler, 1986 |
| Unscheduled DNA synthesis induction | Primary ACI rat hepatocytes | 60 & 600 μM | Negative | Mori *et al.*, 1984 |
| | Primary C3H mouse hepatocytes | 65 & 650 μM | Negative | Mori *et al.*, 1984 |
| DNA breakage | *E. coli* D110 polA | 10, 20, 25 & 50 μg/ml | Positive | Lee & Roschenthaler, 1986 |
| DNA-repair | human or rat liver cells | $1.6x10^{-3}$ - $1.6x10^{-5}$ | Negative | Belitsky *et al.*, 1985 |
| DNA breakage | FM3A mouse mammary carcinoma cells | 1.0, 3.2, 10 μg/ml | Positive | Umeda *et al.*, 1977 |
| DNA breakage | AWRF cells<br>CHO cells | 2 & 10 μg/ml<br>2, 8 & 10 μg/ml | Positive | Stetina & Votava, 1986 |
| DNA synthesis inhibition test | human fibroblasts | $1.0x10^{5}$ | Negative | Yanagisawa *et al.*, 1987 |

**Table 3. Results of *in vivo* genotoxicity assays on Patulin**

| Test system | Test object | Concentration | Result | Reference |
|---|---|---|---|---|
| Chromosome aberrations | Chinese hamster bone marrow | oral 2 x 10 and 20 mg/kg bw | Positive | Roll *et al.*, 1990 |
| Chromosome aberration induction | Chinese hamster bone marrow cells | 2 x 20 mg/kg bw by gavage(6) | Positive | Korted *et al.*, 1979 |
| Chromosome aberration induction | Chinese hamster bone marrow cells | 2 x 1, 10 & 20 mg/kg bw by gavage | Positive | Korte, 1980 |
| Chromosome aberration induction | Chinese hamster bone marrow cells | 2 x 10 & 20 mg/kg bw | Positive | Korte & Ruckert, 1980 |
| Host mediated assay in Swiss albino mice | *S. typhimurium* G46 | i.m. 3 x $<500$ µg | Negative | Gabridge & Legator, 1969 |
| Host mediated assay in male NMRI mice | *S. typhimurium* TA1950, TA1951 | 10 x 20 mg/kg bw gavage | Negative | Von Wright & Lindroth, 1978 |
| Dominant lethal assay | ICR/Ha Swiss | 0.1 & 0.3 mg/kg | Negative | Epstein *et al.*, 1972 |
| Dominant lethal assay | Sprague-Dawley rats | 1.5 mg/kg bw 5x/wk x 10-11 wk, by gavage | Negative | Dailey *et al.*, 1977b |
| Dominant lethal assay | Texas ICR x Sprague-Dawley Sch:Ars(CFI)f | 3.0 mg/kg bw i.p. | Negative | Reddy *et al.*, 1978 |
| Sister chromatid exchange induction | Chinese hamster bone marrow cells | 2 x 1, 10 & 20 mg/kg bw by gavage | Negative | Korte, 1980 |
| Dominant lethal assay | NMRI mice (♂) | i.p. 2.5 and 5 mg/kg bw | Negative | Roll *et al.*, 1990 |

(1) Positive when $CuCl_2$ & NADPH were added
(2) Both with and without S-9 fraction (source not specified)
(3) Both with and without rat liver S-9 fraction
(4) Both with regular plate and preincubation methods
(5) Both with and without mouse liver S-9 fraction
(6) Effect negative if animals first given ethanol as only liquid for 9 wk prior to exposure

### *2.2.9 Special studies on immunotoxicity*

#### *2.2.9.1 In vitro studies*

Peritoneal exudate cells of mice (C57BL/6J) collected by washing the peritoneal cavity, were preincubated for 2 h with 0.01-2 µg patulin/ml. Phagocytosis and phagosome-lysosome fusion were diminished above 0.1 µg/ml, and lysosomal enzymes and microbiological activity above 0.5 µg/ml, whereas $O_2$ production was inhibited only above 2 µg/ml (Bourdiol *et al.*, 1990).

The effects of patulin were investigated on immunological responses of Balb/c mice. *In vitro*, patulin had a stimulatory effect on splenocytes at lower concentration (1 nM to 10 nM) and strongly inhibited lymphocyte proliferation at higher concentrations ($ID_{50}$ from 0.02 to 0.24 $\mu$M depending on mitogens) (Paucod *et al.*, 1990).

At concentrations from 0.25-1 $\mu$g/ml, patulin decreased the chemotactic index of dog neutrophilic granulocytes stimulated by opsonized zymosan. At the same concentrations patulin favoured the migration of the cells. At 1 $\mu$g/ml it inhibited the liberation of superoxide ions by neutrophils, but did not modify their ability to phagocyte *Saccharomyces cerevisiae* even at concentrations up to 10 $\mu$g/ml. The immunosuppressive actions may be explained by a fixation of patulin on sulfhydric groups present on the neutrophil membrane (Dubech, *et al.*, 1993).

In alveolar macrophage harvested from male Long-Evans hooded rats, patulin caused a significant increase in mean cell volume after 2 h exposure at $10^{-3}$ M. Chromium release from alveolar macrophage following exposure to patulin was both time- and concentration-dependent. Treatment with $\geq 1.5 \times 10^{-4}$ M caused significant chromium release within 30 minutes. ATP concentrations in alveolar macrophage monolayer cultures were markedly inhibited within 1 h at concentrations $> 5 \times 10^{-5}$ M patulin. Incorporation of [$^3$H]-precursors into protein and RNA was also strongly inhibited by patulin. Inhibition was both time- and concentration-dependent for both classes of molecules but protein synthesis was sensitive to 10- to 100- fold lower concentrations of patulin than RNA synthesis at the same time interval. The dose producing 50% inhibition at 1 h ($ED_{50}$) was estimated at ca. $1.6 \times 10^{-6}$ M and $1.0 \times 10^{-5}$ M for [$^3$H]-leucine and [$^3$H]-uridine incorporation, i.e. protein and RNA synthesis, respectively. Patulin strongly inhibited phagocytosis of [$^{51}$Cr]-sheep erythrocytes and there was significant inhibition of phagocytosis at $> 5 \times 10^{-7}$ M patulin (Sorenson *et al.*, 1986).

### *2.2.9.2 In vivo studies*

The effects of patulin were investigated on immunological responses of Balb/c mice. In mice, patulin at dose levels of 2 and 4 mg/kg bw significantly reduced delayed type hypersensitivity to *Bordetella pertussis* antigen and did not reduced anti-KLH antibody production (Paucod *et al.*, 1990).

Mice (Swiss female IFA CREDO) receiving 10 mg/kg bw/day patulin for 4 days showed enhanced resistance to i.p. challenge with $10^8$ viable *Candida albicans* at day 2. Immunoglobulin levels (IgA, IgM and IgG) were markedly depressed (10-75%) (Escoula *et al.*, 1988a).

Mice (Swiss female IFA CREDO) were given by gavage 10 mg/kg bw patulin daily from day 0 to day 4, and rabbits received intraperitoneally 2.5 mg/kg bw. The mice were lymphopenic on days 5 and 10, but not on day 20.

There was no effect on neutrophil count on day 5. A significant suppression of the chemilumminescene response of peritoneal leucocytes was observed in both species. Mitogenic responses of mice splenic lymphocytes and rabbit peripheral cells were slightly suppressed (ConA) by treatment with 0.05 μg/ml and markedly inhibited with 0.5 μg/ml. The inhibition was more pronounced on B-cell mitogen compared with T-cell mitogen. In mice and rabbits IgG, IgA and IgM levels obtained on day 5 were lower when treated with patulin (Escoula, *et al.*, 1988b).

Patulin inhibited DNA synthesis in peripheral lymphocytes. These effects were mitigated by cysteine which suggested that sulfhydryl binding was involved in patulin induced toxicity. In mice, an increased resistance to *Candida albicans* was observed and decreased concentrations of circulating immunoglobulin. In rabbits decreased serum immunoglobulin, reduced blastogenesis of lymphocytes and reduced chemiluminescence of peritoneal leucocytes were observed. No details about concentrations were given (Sharma, 1993).

### *2.2.10 Special studies on neurotoxicity*

Groups of 25 albino rats (sex not specified) weighing 25-30 g received 0 or 100 mg of patulin in propylene glycol intraperitoneally on alternate days (dose approximately 1.6 mg/kg bw/day) for 1 month. The patulin-treated animals showed convulsions, tremors, impaired locomotion, stiffness of hindlimbs, and wagging of the head. Patulin inhibited acetylcholinesterase and NaKATPase in the cerebral hemisphere, cerebellum and medulla oblongata. Concomitantly, acetylcholine levels were raised in these brain segments (Devaraj *et al.*, 1982a).

### *2.2.11 Special studies on teratogenicity and embryotoxicity*

#### *2.2.11.1 Mice*

Twelve pregnant Swiss mice received 0 or 2 mg/kg bw/day of patulin in water containing 0.05% lactic acid twice daily by gavage for 6 days starting 14 days after mating. The control mice received 0.05% lactic acid by gavage.

Mean survival time was significantly reduced in the patulin treated dams, while 2/12 control animals and 5/12 experimental animals developed tumours. Of the offspring, 8/43 male and 11/52 female suckling mice died in the first 6 days of life, with hyperemia and bleeding in the brain, lungs and skin. When these early deaths were excluded from the calculations, patulin did not affect survival time in the animals exposed *in utero*. No evidence of carcinogenicity was observed in the offspring exposed only to patulin *in utero* (Osswald *et al.*, 1978).

Groups of 22-31 mice (NMRI) received orally during days 12 and 13 of gestation 0 or 3.8 mg/kg bw/day, or i.p. 0, 1.3, 2.5 or 3.8 mg/kg bw/day. Higher dose levels were maternally toxic. Oral administration caused no effects

on the number of implantation, delivered fetuses, number of resorption, dead fetuses, fetal weight, or malformations of the skeleton and organs. Intraperitoneal administration showed at 3.8 mg/kg bw/day a slight increase in early resorption, compared to controls. An increase in cleft palate was seen (10.6% compared to 1.5% in controls), and an increase in malformations of the kidney (2.8% compared to none in the control group) were also seen at this dose level (Roll *et al.*, 1990).

#### *2.2.11.2 Rats*

In a 2-generation reproductive toxicity study (see section 2.2.4.1), offspring of 15 Sprague-Dawley dams of the $F_1$ and $F_2$ generation exposed by gavage to 0 or 1.5 mg/kg bw/day of patulin in citrate buffer were evaluated for teratological abnormalities. Patulin caused an increase in resorption in the $F_1$ litters, but this effect was not observed in the $F_2$ generation. The average weight of male fetuses of the $F_2$ generation was significantly less than controls. No increase in skeletal or soft tissue abnormalities was observed (Dailey *et al.*, 1977b).

However, when patulin was administered i.p. to groups of 10-17 pregnant Charles River CDI rats at doses of 1.5 or 2.0 mg/kg bw/day, a significant decrease in average fetal body weight was observed at the lower dose, and at 2.0 mg/kg bw/day all implanted embryos were resorbed (Reddy, *et al.*, 1978).

#### *2.2.11.3 Chickens*

Patulin was injected into the air cell of chick eggs. It was reported to be embryotoxic at levels of 2.4-69 μg/egg depending on the age of the embryo, and teratogenic at levels of 1-2 μg/egg. Patulin/cysteine adducts exhibited the same toxic effects, but at much higher doses: 15-150 μg of patulin equivalents (Ciegler *et al.*, 1976).

#### *2.2.11.4 In vitro studies*

Whole rat embryo culture was used to determine the teratogenic potential of patulin *in vitro*. Embryos were exposed to untreated or patulin-treated (0 - 62 μM) rat serum for 45 h. The embryos exposed to 62 μM patulin were not evaluated because they did not survive the 40 h incubation time. The results indicated that patulin induced a concentration-dependent reduction in protein and DNA content, yolk sac diameter, crown rump length, and somite number count. Patulin treatment also resulted in an increase in the frequency of defective embryos. Anomalies included growth retardation, hypoplasia of the mesencephalon and telencephalon, hyperplasia and/or blisters of the mandibular precess (Small *et al.*, 1992, summary only).

### *2.3 Observations in humans*

Patulin was tested as an antibiotic for treatment of the common cold in humans. Application was through the nasal route (1:10 000 or 1:20 000 solutions, every 4 h). Most of the information is anecdotal (Gye, 1943).

A report on a controlled trial failed to identify the number of patients tested, and was unclear as to which clinical tests were performed to support the authors assertion that no ill effects were observed (Hopkins, 1943).

## *3. COMMENTS*

In rats, most of the administered dose was eliminated within 48 h in faeces and urine, less than 2% being expired as carbon dioxide. No other metabolites have been identified. About 2% of the administered dose was still present after 7 days, located mainly in erythrocytes.

Patulin has a strong affinity for sulfhydryl groups, which explains why it inhibits the activity of many enzymes. Patulin adducts formed with cysteine were less toxic than the unmodified compound in acute toxicity, teratogenicity, and mutagenicity studies.

In acute and short-term studies, patulin caused gastrointestinal hyperaemia, distension, haemorrhage and ulceration. Pigtail monkeys (*Macaca nemestrina*) tolerated patulin consumption of up to 0.5 mg/kg bw/day for 4 weeks without adverse effects.

The NOEL in a 13-week toxicity study performed in rats was 0.8 mg/kg bw/day, based on a slight impairment of kidney function and a villous hyperaemia in the duodenum in the mid- and high-dose groups.

Two reproductive toxicity studies in rats and teratogenicity studies in mice and rats were available. No reproductive or teratogenic effects were noted in mice or rats at dose levels of up to 1.5 mg/kg bw/day. However, maternal toxicity and an increase in the frequency of fetal resorptions were observed at higher levels, which indicated that patulin was embryotoxic.

Both *in vitro* and *in vivo* experiments indicated that patulin had immuno-suppressive properties. However, the dose levels at which these effects occurred were higher than the NOEL in both the short-term toxicity study and a combined reproductive toxicity/long-term toxicity/carcinogenicity study.

Although the data on genotoxicity were variable, most assays carried out with mammalian cells were positive while assays with bacteria were mainly negative. In addition, some studies indicated that patulin impaired DNA synthesis. These genotoxic effects might be related to its ability to react with sulfhydryl groups and thereby inhibit enzymes involved in the replication of

genetic material. Nevertheless, it was concluded from the available data that patulin is genotoxic.

The mortality seen in short-term toxicity, reproductive toxicity and long-term toxicity studies with conventional rats, due to dilatation of the gut and/or pneumonia, was most probably secondary to the fact that patulin acts like an antibiotic on Gram-positive bacteria, thereby giving a selective advantage to pathogenic Gram-negative bacteria. This conclusion was supported by the fact that, in 13-week studies at similar dose levels with specific pathogen-free (SPF) rats, no such mortality was seen.

In combined reproductive toxicity, long-term toxicity/ carcinogenicity study in rats, a dose level of 0.1 mg/kg bw/day of patulin produced no effect in terms of decreased weight gain in males. However, as patulin was administered only three times per week during 24 months, the NOEL derived from this study was 43 $\mu$g/kg bw/day.

An additional long-term carcinogenicity study in a rodent species other than the rat, which was recommended at the previous meeting for the further evaluation of the toxicity of patulin, was not available.

## *4. EVALUATION*

Since in the most sensitive experiment, patulin was administered only three times per week, the existing PTWI was changed. As it does not accumulate in the body and in the light of the consumption pattern, the PTWI was changed to a provisional maximum tolerable daily intake (PMTDI). Based on a NOEL of 43 $\mu$g/kg bw/day and a safety factor of 100, a PMTDI of 0.4 $\mu$g/kg bw was established.

Submission of the results of a long-term toxicity/carcinogenicity study in a rodent species other than the rat is desirable.

Patulin levels in apple juice are generally below 50 $\mu$g/litre and maximum intakes have been estimated to be 0.2 $\mu$g/kg bw/day for children and 0.1 $\mu$g/kg bw/day for adults, i.e. well below the tolerable intake established by the Committee. However, apple juice can occasionally be heavily contaminated and continuing efforts are therefore needed to minimize exposure to this mycotoxin by avoiding the use of rotten or mouldy fruit.

## *5. REFERENCES*

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