EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Forty-fourth report of the
Joint FAO/WHO Expert Committee on
Food Additives

can also occur in meat and dairy products derived from animals exposed to ochratoxin A-contaminated feedstuffs.

Ochratoxin A was first evaluated at the thirty-seventh meeting of the Committee (Annex 1, reference 94), when a provisional tolerable weekly intake (PTWI) of 112 ng per kg of body weight was established. The assessment was based on the deterioration of renal function in pigs, for which the lowest-observed-effect level was 0.008 mg per kg of body weight per day (a no-effect level was not observed). A safety factor of 500 was used in deriving the tolerable intake of ochratoxin A. At that time, the Committee recommended that efforts should be made to highlight the need for ensuring proper storage conditions for grain and grain products. Furthermore, appropriate ochratoxin A residues should be monitored to obtain better estimates of dietary exposure and to identify populations at greater risk with a view to implementing preventive measures. The Committee also encouraged further studies aimed at elucidating the role of ochratoxin A and other mycotoxins in nephropathy in pigs and humans, the mechanism of induction of tumours, and the role of phenylalanine in antagonizing the adverse effects of ochratoxin A.

In view of the increasing number of reports on the occurrence of ochratoxin A in food commodities in several countries, the Committee was asked to re-evaluate this substance.

Since the last review a number of toxicological studies have been conducted, including investigations on epidemiology, genotoxicity and nephrotoxicity. Although the results of these studies are important for understanding the biological effects of ochratoxin A, the Committee did not consider that they justified any change in the basis on which the previous assessment of the tolerable intake of ochratoxin A was made. In addition, the Committee confirmed that nephrotoxicity was the most sensitive effect of ochratoxin A and that the increased incidence of both benign and malignant tumours seen in the rat occurred at higher doses.

The Committee reconfirmed the PTWI established at the thirty-seventh meeting, rounded it off to 0.1 µg per kg of body weight, and reiterated its request for further studies on ochratoxin A.

The Committee noted that grain should be stored under suitable conditions to keep levels of ochratoxin A to a minimum.

An addendum to the toxicological monograph was prepared.

3.3.2 *Patulin*

Patulin is a mycotoxin produced by certain species of the genera *Aspergillus* and *Penicillium*, including *A. clavatus*, *P. expansum*, *P. patulum*, *P. aspergillus* and *P. byssochlamys*. *P. expansum* is a common spoilage microorganism in apples, and the major potential dietary sources of patulin are apples and apple juice made from affected fruit.

Patulin was previously evaluated by the Committee at its thirty-fifth meeting (Annex 1, reference *88*), when a PTWI of 7 μg per kg of body weight was established, based on a no-effect level of 0.1 mg per kg of body weight per day in a combined reproductive toxicity/long-term toxicity/carcinogenicity study in rats. Additional information has become available since the last evaluation.

Patulin was reviewed by the International Agency for Research on Cancer in 1976 and 1985 (*17, 18*). It was concluded at the second of these reviews that there was inadequate evidence for carcinogenicity of patulin in experimental animals. No evaluation could be made of carcinogenicity of patulin in humans.

In rats, most of the administered dose was eliminated within 48 hours 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 per kg of body weight per day for 4 weeks without adverse effects.

The NOEL in a 13-week toxicity study performed in rats was 0.8 mg per kg of body weight per 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 per kg of body weight per 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 the combined reproductive toxicity/long-term toxicity/carcinogenicity study in rats, a dose level of 0.1 mg per kg of body weight per 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 µg per kg of body weight per 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.

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 µg per kg of body weight per day and a safety factor of 100, a PMTDI of 0.4 µg per kg of body weight 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 µg per litre, and maximum intakes have been estimated to be 0.2 µg per kg of body weight per day for children and 0.1 µg per kg of body weight per 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.

A toxicological monograph, summarizing both the information given in the previous toxicological monograph and information received since the previous review, was prepared.

4. Revision of certain specifications

4.1 General

A total of 34 substances were examined for specifications only (see Annex 2). For 13 of these substances, no information was received to support the re-evaluation. The Committee was, however, able to revise the existing specifications for 31 substances, based on the substantial

PATULIN

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1. EXPLANATION

Patulin is a mycotoxin produced by certain species of the genera *Aspergillus* and *Penicillium*, including *A. clavatus*, *P. expansum*, *P. patulum*, *P. aspergillus* and *P. byssochlamys*. *P. expansum* is a common spoilage microorganism in apples, and the major potential dietary sources of patulin are apples and apple juice made from affected fruit.

Patulin was previously evaluated by the Committee at its thirty-fifth meeting (Annex 1, reference 88), when a PTWI of 7 μg/kg bw was established based on a no-effect level of 0.1 mg/kg bw/day in a combined reproductive toxicity/long-term toxicity/carcinogenicity study in rats. Additional information has become available since the last evaluation.

Patulin was reviewed by IARC (IARC, 1976; 1985). It was concluded at the second of these reviews that there was inadequate evidence for carcinogenicity of patulin in experimental animals. No evaluation could be made of carcinogenicity of patulin in humans.

The following toxicological monograph summarizes both the information given in the previous toxicological monograph and information received since the previous review.

2.   BIOLOGICAL DATA

2.1   Biochemical aspects

2.1.1   Absorption, distribution, and excretion

A single oral dose of 3 mg/kg bw of ^{14}C-patulin in citrate buffer was given to 17 male and 12 female Sprague-Dawley rats exposed for 41-66 weeks after birth to levels of 0 or 1.5 mg/kg bw of patulin in 1 mol/litre citrate buffer. All animals were fasted for 24 h before the administration of the labelled patulin. Animals were placed in metabolic cages and faeces, urine and CO_2 were collected. One or 2 animals/sex/group (untreated or pretreated with patulin) were sacrificed at 4, 24, 48, 72 h or 7 days after blood was collected for patulin determination. Concentrations of patulin in erythrocytes were calculated from the difference between radioactivity of whole blood and serum. Within 7 days, 49% and 36% of the administered radioactivity was recovered in faeces and in urine, respectively. Most of the excretion of label occurred within the first 24 h. Approximately 1-2% of the label was recovered as ^{14}CO$_2$. At the end of 7 days, 2-3% of the radioactivity was recovered in soft tissues and blood. The major retention sites of patulin were erythrocytes and blood-rich organs (spleen, kidney, lung and liver) (Dailey *et al.*, 1977a).

2.1.2   Effects on enzymes and other biochemical parameters

In vivo studies

Absorption of radiolabelled glycine, alanine and lysine was reduced in perfused intestines of rats that had received 100 mg of patulin intraperitoneally on alternate days for 1 month (equal to 1.6 mg/kg bw/day). The authors attributed this effect to reduced total ATPase, NaK ATPase, and alkaline phosphatase activities which were studied in a satellite group of rats (Devaraj *et al.*, 1982a).

A group of albino rats received 0.1 mg of patulin in 0.2 ml of propylene glycol, injected i.p. on alternate days. A control group was treated similarly with propylene glycol alone. The animals were sacrificed after 15 doses. Liver, kidney and intestine were used for the assay of various enzymes such as glycogen phosphorylase, hexokinase, glucose-6-phosphatase, fructose-1,6-diphosphorylase, hexokinase, glucose-6-phosphatase, fructose-1,6-diphosphatase and aldolase.

The concentration of aldolase in the liver, kidney and intestinal tissue was reduced during patulin toxicosis. In a follow up experiment, groups of rats were treated similarly and the rats were sacrificed after 20 doses. Aldolase was

isolated and purified and studies on its kinetic properties were made. These studies did not show any significant variations in the properties of liver aldolase of normal and patulin-treated rats. The authors concluded that the results suggested that patulin toxicosis inhibited the biosynthesis of liver aldolase (Sakthisekaran & Shanmugasundaram, 1990).

Forty-eight hours after i.p. injection of 5.0 or 7.5 mg/kg bw of patulin in male ICR mice, NaKATPase and MgATPase of liver, kidney and brain preparations were significantly inhibited. Injection of 2.5 mg/kg bw had no significant effect on enzyme activity. The same effects were demonstrated in *in vitro* studies with mitochondrial and microsomal fractions of liver, kidney and brain of ICR mice (Phillips & Hayes, 1977).

Patulin inhibited acetylcholinesterase and NaKATPase in cerebral hemisphere, cerebellum and medulla oblongata in rats treated for 1 month with i.p. injections of 1.6 mg/kg bw/day patulin. Concomitantly, acetylcholine levels were raised in these brain segments (Devaraj *et al.*, 1982b).

A non-competitive and irreversible inhibition of the activity of alcohol dehydrogenase derived from yeast was attributed to patulin's ability to bind to SH-groups; the K_i was found to be 5.0×10^{-5} M (Ashoor & Chu, 1973a).

Inhibition of yeast-derived aminoacyl-tRNA synthetase by patulin was mainly due to modification of the enzyme's sulfhydryl groups (Arafat, *et al.*, 1985).

Liver lactate dehydrogenase was increased in 4 pregnant Sprague-Dawley rats after exposure by gavage to 3 mg/kg bw/day of patulin in tris-acetate buffer, from days 1-19 of gestation (Fuks-Holmberg, 1980).

Malate dehydrogenase in human placental microsome- and mitochondria-rich fractions was increased up to 15 times when incubated with 0.5 - 3 mg/g placenta of patulin *in vitro* (Fuks-Holmberg, 1980).

Placental GPT was depressed in 4 pregnant Sprague-Dawley rats after exposure by gavage to 3 mg/kg bw/day of patulin in tris-acetate buffer, from days 1-19 of gestation (Fuks-Holmberg, 1980).

When white male albino mice were injected with 10 doses of 0.1 mg of patulin in propylene glycol on alternate days, glycogen phosphorylase in the liver was activated, and blood glucose levels increased by 60%. These results were confirmed by *in vitro* studies (Madiyalakan & Shanmugasundaram, 1978).

Groups of 10 rats were fed regular diet, diet infected with *Penicillium patulum*, or received i.p. injections of purified patulin (1 mg/kg bw on alternate days) for 3 months. Fasting blood glucose levels were elevated and a glucose tolerance test revealed an elevated glucose curve and reduced insulin

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production. The authors concluded that patulin was diabetogenic (Devaraj *et al.*, 1986).

Male F344 rats received a single i.p. injection of 0, 0.5, 5 or 10 mg/kg bw patulin. Liver mixed function oxidase and cytochrome P-450 activity were determined 4 days after treatment. Oxidative cleavage of phosphonothioate and aryl hydrocarbon hydroxylase were elevated at 10 mg/kg bw. No effect was observed on p-nitroanisole O-demethylase or on cytochrome P-450 (Kangsadalampai *et al.*, 1981).

Patulin was reported to induce mixed function oxidase in male ICR mice treated with 0.5, 1.0 or 2.0 mg/kg bw of patulin intraperitoneally (Siraj & Hayes, 1978).

Patulin inhibited protein prenylation in mouse FM3A cells. An inhibition of 50% and 80% was observed at 7 μM and 100 μM, respectively. Protein synthesis, as measured by the incorporation of ^{14}C-leucine, was also inhibited by patulin. The inhibition was 50% at 3 μM and >90% at 30 μM. In a cell-free assay, patulin inhibited rat brain farnesyl protein transferase, one of the enzymes responsible for protein prenylation. The inhibition was 50% at a concentration of 290 μM (Miura, *et al.*, 1992).

The concentration of glycogen in liver, kidney and intestinal tissues was reduced during patulin toxicosis. The decrease in hepatic glycogen indicated glucose intolerance which may be due to insulin insufficiency. This may be reflected in decreased concentration of insulin-dependent enzymes. Glycogen phosphorylase was markedly increased, while glycolytic enzymes such as hexokinase and aldolase were significantly lowered. Gluconeogenesis was stimulated as evidenced by increased glucose-6-phosphatase and fructose-1,6-diphosphatase activity (Sakthisekaran *et al.*, 1989).

In vitro studies

Oxygen uptake stimulated by Krebs-cycle intermediates was reported to be inhibited in tissue extracts from mice, rats and golden hamsters. Inhibition of oxygen uptake in liver homogenates was observed at levels of patulin as low as 0.033 mM. Inhibition of oxygen uptake in heart and muscle homogenates was greater than in liver homogenates. Patulin competitively inhibited succinate dehydrogenase in mouse liver homogenates. The P/0 ratio was not affected by the toxin. In comparative studies, the golden hamster was more susceptible, and the rat less susceptible to patulin inhibition than the mouse (Hayes, 1977).

Kidney explants from male Osborne-Mendel rats, when incubated for 18 h in media containing 0.5, 0.75, or 1.0 mM patulin *in vitro*, lost their respiratory ability as measured by conversion of ^{14}C-glucose to ^{14}CO$_2$. During measurement of respiration, patulin was not present in the reaction mixture. At 1.0 mM patulin, respiration was increased. Leakage of protein into the medium

at a concentration of 1.0 mM patulin may indicate increased cell membrane permeability (Braunberg *et al.*, 1982).

Patulin inhibited the *in vitro* activity of NaKATPase in microsomes prepared from mouse brain. Activity was partially restored by washing. Preincubation of patulin with dithiothreitol or glutathione prevented the inhibition (Phillips & Hayes, 1978).

Non-competitive inhibition was demonstrated when patulin was incubated with rabbit-muscle aldolase; the K_i was 1.3 x 10^{-5} M. The cysteine adduct of patulin was a less effective inhibitor (Ashoor & Chu, 1973b).

Patulin, at a level of 4.35 μmol/ml, was reported to inhibit by 29% and 84% the activity of DNA-dependent RNA polymerase I and II prepared from rat liver nuclei (Tashiro *et al.*, 1979).

Patulin at a level of 200 μg/ml inhibited *in vitro* the chain initiation stage of RNA synthesis in rat liver nuclei (Moule & Hatey, 1977).

Ribonuclease H, prepared from rat liver nuclei, was inhibited by patulin *in vitro* by 62% at a concentration of 0.32 μmol/mol, and by 47% at a concentration of 1.07 μmol/ml (Tashiro *et al.*, 1979).

Acid RNAse in human placental microsome and mitochondria-rich fractions was increased up to 1.5 times when incubated with 0.5-3 mg patulin/g of placenta *in vitro* (Fuks-Holmberg, 1980).

Patulin caused a competitive inhibition of lactate dehydrogenase from rabbit muscle (K_i = 7.2 x 10^{-5} M). The presence of cysteine reversed the inhibitory effect of patulin on lactate dehydrogenase (Ashoor & Chu, 1973a).

2.2   *Toxicological studies*

2.2.1   *Acute toxicity studies*

The acute toxicity of patulin is summarized in Table 1. Toxic signs consistently reported in all studies were agitation, in some cases convulsions, dyspnea, pulmonary congestion and edema, and ulcerations, hyperemia and distension of the GI tract.

Acute toxicity of i.p. administered patulin was reported to be reduced by simultaneous administration of another mycotoxin, rubratoxin B (Kangsadalampai *et al.*, 1981).

When a patulin/cysteine adduct was administered to mice intraperitoneally, no acute toxicity was observed at levels up to 150 mg of patulin/mouse (Ciegler *et al.*, 1976).

Table 1. Acute toxicity of patulin

Species	Sex	Route	LD$_{50}$ (mg/kg bw)	References
Mouse	M	oral	29-48	Escoula, *et al.*, 1977 Lindroth & von Wright, 1978
	F	oral	46.31	McKinley & Carlton, 1980a
	M&F	"	17	Hayes *et al.*, 1979
	?	"	25	Katzman *et al.*, 1944
	M	i.p.	5.7-8.17	Ciegler *et al.*, 1976 Escoula *et al.*, 1977 McKinley & Carlton, 1980a
	F	i.p.	10.85	Escoula *et al.*, 1977
	M&F	"	7.6	Hayes *et al.*, 1979
	?	"	4-5.7	Katzman *et al.*, 1944 Ciegler *et al.*, 1976
	M&F	i.v.	8.57	Escoula *et al.*, 1977
	?	s.c.	8-10	Katzman *et al.*, 1944
	M	s.c.	10	McKinley & Carlton, 1980a
Rat	M	oral	30.53-55.0	Escoula *et al.*, 1977 McKinley *et al.*, 1982
	F	oral	27.79	Escoula *et al.*, 1977
	?	"	32.5	Dailey *et al.*, 1977b
	M&F	"	108-118	Hayes *et al.*, 1979
	M	i.p.	4.59-10.0	Escoula *et al.*, 1977 McKinley *et al.*, 1982
neonatal rats	F	oral	5.70	Escoula *et al.*, 1977
	M&F	"	6.8	Hayes *et al.*, 1979
weanling rats	M&F	i.p.	5.9	Hayes *et al.*, 1979
	M	i.v.	8.57	Escoula *et al.*, 1977
	M	s.c.	11.0	McKinley *et al.*, 1982
	?	s.c.	25	Katzman *et al.*, 1944
Hamster	M	oral	31.5	McKinley & Carlton, 1980b
		i.p.	10	McKinley & Carlton, 1980b
		s.c.	23	McKinley & Carlton, 1980b

2.2.2   *Short-term toxicity studies*

2.2.2.1   *Mice*

When patulin was administered by gavage in citrate buffer to groups of 10 male Swiss ICR mice at doses of 0, 24 or 36 mg/kg bw, daily or on alternate days for 14 days, body weight was depressed and mortality was increased in a dose-dependent manner. Histopathological lesions were found in the GI tract, which included epithelial degeneration, haemorrhage, ulceration

of gastric mucosa, and exudation and epithelial desquamation in the duodenum (McKinley & Carlton, 1980a).

2.2.2.2 Rats

When patulin was administered by gavage to groups of 10 male Sprague-Dawley rats at doses of 28 or 41 mg/kg bw, daily or on alternate days for 14 days, initial loss of body weight was observed; animals recovered after day 4. Mortality was increased in all treated groups, but no dose dependency was observed. Gross lesions were found in the stomach and small intestine; the gastric mucosa was reddened and the stomach was distended. The duodenum and jejunum were distended by fluid. Histopathological lesions were found in the stomach which consisted of ulceration of the mucosa, epithelial degeneration, haemorrhage, and neutrophil and mononuclear cell infiltration (McKinley *et al.*, 1982).

Drinking-water containing 0, 25, 85, or 295 mg/litre of patulin in 1 mM citrate buffer was given to groups of 6 SPF RIVM:Tox (Wistar-derived) rats for 4 weeks. Food and liquid intake were recorded three times per week. Body weights were determined at the start of the experiment and at termination. Urinalysis, including urine volume, bilirubin, and urinary protein were determined in the last week. Creatinine clearance was calculated from serum and urine levels of creatinine. At termination, the animals were examined macroscopically, and the liver, spleen, thyroid glands, brain, kidneys, heart, mesenteric lymph nodes, adrenal glands, thymus, testes and ovaries were weighed. Histopathological examination was carried out on all organs and tissues of the high-dose and the control groups. Food and liquid intake were reduced in the mid- and high-dose animals. Body weights at the high-dose level were decreased. Creatinine clearance was lower in the high-dose animals, but no morphological glomerular damage was observed. In the high-dose group, fundic ulcers in the stomach were observed in combination with enlarged and active pancreatico-duodenal lymph nodes, while villous hyperemia of the duodenum was observed at the mid- and high-dose levels. The authors suggested, based on normal appearance of the adrenal glands, that the observed effects in the GI tract were a direct effect of patulin on the tissue, which was not mediated through adrenal gland stimulation (stress). The NOEL in this study was 25 mg/litre (Speijers *et al.*, 1985, 1988).

Albino rats were given 0.1 mg patulin in 0.1 ml propylene glycol injected intraperitoneally on alternate days. Control rats were treated with propylene glycol only. The animals were sacrificed after 15 doses. Blood was collected and liver, kidney and intestine were used for the estimation of DNA and RNA. In plasma, the total protein level, albumin concentration and A/G ratio were significantly decreased in the dosed animals. The levels of DNA and RNA in liver, kidney and intestine were significantly reduced (Gopalakrishnan & Sakthisekaran, 1991).

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Twenty rats/sex received by gavage 0 or 0.1 mg/kg bw patulin dissolved in water on alternate days for 30 days. At the end of the 30th day, rats were killed and the intestine was removed and used for the estimation of lipids and Na^+-K^+ dependent ATPase.

Total lipids (25.8%), phospholipid (20.6%) and triglycerides (59.8%) decreased significantly whereas total cholesterol levels showed a slight increase (12.6%) in the experimental rats. A marked inhibition of Na^+-K^+ dependent ATPase (32.4%) was observed in the intestines of experimental rats (Devaraj & Devaraj, 1987).

Groups of Wistar rats (10/sex/group) were given drinking-water containing patulin at concentrations of 0, 6, 30 or 150 mg/litre in 1mM citrate buffer for 13 weeks. Food and water intake were decreased in the mid- and high-dose groups. Body-weight gain was decreased only in animals of the high-dose group. Haematological parameters were slightly altered in the high-dose group. No effect of patulin on the intestinal microflora was observed. A slight impairment of the kidney function and a villous hyperaemia in the duodenum in the mid- and high-dose groups were observed. The NOAEL in this study was 6 mg/litre drinking-water, equivalent to 0.8 mg patulin/kg bw/day (Speijers, *et al.*, 1986).

2.2.2.3   Hamsters

When patulin was administered by gavage to groups of 10 male Syrian golden hamsters at doses of 0, 16 or 24 mg/kg bw, daily or on alternate days for 14 days, loss of body weight was observed and mortality was increased in all treated groups, but no dose dependency was observed. Gross lesions were found in the stomach and duodenum. Histopathological lesions were found in the GI tract and included epithelial degeneration, haemorrhage and ulceration (McKinley & Cariton, 1980b).

2.2.2.4   Chickens

Fifteen one-day old white leghorn chicks were given by intubation 0 or 100 μg patulin, every 48 h. At the end of the 15th dose, the birds were fasted overnight and killed. Kidney and intestine were removed and assayed. The experimental chicks showed reduced enzyme activity. The reduction in the activity of total ATPase in the kidney was 40% and in the intestine 52% while the reduction in the Na^+-K^+ dependent ATPase activity in the kidney was 46% and in the intestine 55% (Devaraj *et al.*, 1986).

2.2.2.5   Monkeys

Groups of 1 male and 1 female pigtail monkeys *(Macaca nemestrina)* received doses of 0, 0.005, 0.05, or 0.5 mg/kg bw/day of patulin for 4 weeks. Monkeys of the highest dose group received 5 mg/kg bw/day patulin for 2 additional weeks. Weekly determinations were made of SGOT, SAP, BUN,

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