

## THE TOXINS OF *BYSSOCHLAMYS NIVEA* WESTLING I. ACUTE TOXICITY OF PATULIN IN ADULT RATS AND MICE

ESCOULA L., MORÉ J. and BARADAT C.

with the technical assistance of Geneviève HENRY and Nicole BRUNEL-DUBECH

Station de Pharmacologie-Toxicologie, I.N.R.A. 180, chemin de Tournefeuille, 31300 TOULOUSE.

### Résumé

LES TOXINES DE *BYSSOCHLAMYS NIVEA* WESTLING. 1. TOXICITE AIGUE DE LA PATULINE CHEZ DES RATS ET SOURIS ADULTES. — La toxicité aiguë de la patuline est mesurée chez le rat et la souris adultes des deux sexes par les voies orale, intraveineuse et intrapéritonéale. Les  $DL_{50}$  pour les rats sont respectivement de 30,53, 8,57, 4,59 mg/kg chez les mâles et de 27,79 et 5,70 mg/kg par voie orale et intrapéritonéale chez les femelles. Pour la souris, les mêmes  $DL_{50}$  sont de 36,42, 17,80, 8,17 mg/kg chez les mâles et de 46,31, 17,80 et 10,85 mg/kg chez les femelles.

La souris apparaît plus résistante que le rat et les administrations orale et intraveineuse moins toxiques que l'injection intrapéritonéale. Les symptômes et les résultats de l'autopsie sont similaires chez les deux espèces : prostration, douleurs abdominales, difficultés locomotrices du train postérieur, signes respiratoires, cyanose des extrémités, pilo-érection, congestion du tube digestif avec exsudat péritonéal et parfois pleural. Le poumon, congestionné et œdémateux, présente parfois des pétéchies.

Les observations histologiques soulignent l'importance des phénomènes congestifs et hémorragiques qui atteignent la plupart des organes étudiés.

Aux doses maxima tolérées et minima mortelles on note une très légère influence de la toxine sur l'évolution pondérale des animaux. Après l'administration les animaux récupèrent leur poids initial en deux à trois jours.

### Introduction

In previous investigations, we detected patulin in the exploited silo layers, most often infested with *Byssochlamys nivea* Westling (Escoula, 1974). After having specified the conditions of production of the mycotoxin by this fungal species and testing the strains in our possession (Escoula, 1975c), we ensured our own production of

patulin in order to effect the toxicity assays.

The toxic properties of patulin are mainly known in the mouse. Nevertheless, considerable differences are to be noted concerning the determination of the  $LD_{50}$  in this species, particularly according to the administration routes. Thus, with intravenous route, the results vary from 25 mg/kg (Broom *et al.*, 1944) to 15.6 mg/kg (Yamamoto, 1954). Similarly, for intraperitoneal route, the  $LD_{50}$

varies from 30 mg/kg (Andraud *et al.*, 1964) to 15 mg/kg to (Capitaine and Balouet 1974 ; Broom *et al.* 1944), or 5 mg/kg according to the Merck Index (1968).

The purpose of this study is to evaluate the toxicity of patulin both in the mouse and in the rat, by intravenous or intraperitoneal administration but also *per os* which is the natural absorption route of the mycotoxin.

### Material and methods

**Animals :** all experimental subjects were conventionally raised at the Station. Wistar rats had an average body weight of  $157 \pm 5.6$  g for males, and  $153 \pm 4.2$  g of females. Swiss strain mice had an average weight of  $28.7 \pm 0.9$  g for males and  $22.4 \pm 1.1$  g for females. In each species 9 animals were used per dose and per route of administration.

**Environment :** the ambient temperature was regulated at  $21 \pm 1^\circ\text{C}$  and the humidity maintained at  $55 \pm 5\%$ . After randomization of the treatments, the animals were housed 4 or 5 per cage for the rats and 9 for the mice. Water and food were available *ad lib*. Rats received UAR A04 (2900 cal./kg) and mice received UAR A03 (3200 cal./kg).

**Patulin administration :** three routes of administration were employed.

- *per os* (P. OS) : by stomach tubing.
- intraperitoneal (I.P.).
- intravenous (I.V.) : injection into the dorsal vein of the tail, only in both sexes of the mouse.

Logarithmically equidistant doses were in the range of 2 to 45.28 mg of patulin per kg body weight for rats, and 2.85 to 90.56 mg per kg for mice.

The toxin was dissolved in isotonic sodium chloride (0.9 %). According to the parenteral or oral routes, the toxin was administered in volumes of 0.5 or 0.75 ml/250 g body weight for the rat and 0.4 ml per 20 g body weight for the mouse.

**Weighting of the animals :** the animals' weight was noted daily during the 10 days following patulin administration. For each group of animals, the percentage  $(P_n \times 100)/P_o$  was calculated daily, where  $P_o$  represents the weight on the day of administration and  $P_n$  the weight  $n$  days later.

**Histological techniques :** the organs observed were subjected to the following histochemical reaction.

- hemalun-eosin-orange G ;
- Masson's trichrome for anatomotopographic studies ;
- Mallory's hematoxylin phosphotungstic acid for microthrombosis detection ;
- periodic acid-Schiff reagent for the demonstration of polysaccharides rich in free 1-2 glycol radicals (blood cells glycogen).

**Analysis of the results :** For  $LD_{50}$  calculations after 10 days of observations, we employed the probit method. This leads, on one hand, to the regression equations of the form  $y = ax + b$ , where  $y$  represents the probit of mortality and  $x$  represents the logarithm of the dose ; this method also yields the  $LD_{50}$  value and the estimation of its precision. A computer treatment of these data was proposed by Boniface *et al.* (1972) which enables the use of the probit method ; this program was utilized to analyse our results and is available, translated in Fortran, at the *Station de Biométrie*, INRA, Toulouse, France.

### Results

#### 1. Acute toxicity in the rat

##### 1. — 1. Symptomatology.

General symptomatology was practically the same regardless of the means of administration of the toxin or the sex of the animal. The symptoms were more pronounced and their chronology more rapid with increasing doses.

Animals which received the highest toxin concentrations exhibited, beginning several minutes after administration, a phase of intense agitation and signs of abdominal pain (hollowing of the flanks ; the animal permanently sought a sedative position). Afterwards, during the first hour, neuromuscular and respiratory symptoms appeared, the former manifested as :

- a drop in muscle tone (flaccid abdomen) ;
- locomotory difficulties, especially at the level of the hind quarter, leading to a sluggishness of movement. In some extreme cases, the animals drag their hind legs.

The respiratory symptoms were evidenced by a dyspneic respiration, which was gasping and noisy, accompanied by a mucosal discharge. A cyanosis of the body endings (paws, tail, ears) was observed. The spontaneous activity disappeared after one hour and the treated animals, contrary to the controls, were dispersed in the cage. The prostration increased with time and was accompanied by various signs : piloerection, hypothermy, arched back, distended abdo-

TABLEAU 1 TABLE 1  
 Résultats des observations histologiques (Rats)  
*Histological results (Rats)*

Lésions	Rein <i>Kidney</i>	Foie <i>Liver</i>	Poumon <i>Lung</i>	Estomac <i>Stomach</i>	Rate <i>Spleen</i>	Jéjunum <i>Jejunum</i>
Congestion <i>Hyperemia</i>	+ + +	+ + +	+ + +	—	+ +	+
Hémorragies <i>Hemorrhages</i>	+ + +	+ +	+ +	+	+	+
Anomalies nucléaires <i>Nuclear abnormalities</i>	—	Pycnose caryorrhexie <i>Pycnosis caryorrhexis</i>	Hyperchromie <i>Hyperchromia</i>	Pycnose <i>Pycnosis</i>	—	—
Nécrose <i>Necrosis</i>	+	—	—	—	+	—
Ulcerations <i>Ulcers</i>	—	—	—	+	—	+ +
Divers <i>Micellaneous</i>	Cylindres granuleux hyalins densification des floculus <i>Eosinophilic hyaline casts</i>  <i>densification of floculus</i>	Foyers de dégénérescence graisseuse prolifération des cellules de Kupffer <i>Adipous degeneration foci Proliferation of Kupffer cells</i>	Atélectasie hyperplasie des formations lymphoïdes <i>Atelectasia Lymphoid hyperplasia</i>			Invasion constante de la muqueuse par des poly- nucléaires éosinophiles  <i>Infiltration of mucosa by eosinophilic leukocytes</i>

L'intensité des lésions est exprimée par des signes allant de :

+ + + (lésion sévère) à — (pas de lésion).

*Lesions degrees are expressed by drawing from :*

+ + + (*severe lesion*) to — (*no-lesion*).

men, freely flowing tears, ptosis.

Tonic convulsions preceded death, which occurred in an asphyxiated state.

1. — 2. Survival time.

The length of survival depended on the dose and the way of administration : 3h to 2 days for intravenous administration, 4h to 6 days for intraperitoneal introduction and 6h to 6 days for oral administration.

1. — 3. Macroscopic lesions.

Autopsied animals revealed a congestion of subcutaneous connective tissue. When opening the peritoneal cavity, a significant

exudate was observed ; the digestive apparatus, the liver, the spleen and the kidneys were congested. In the pleural cavity, the exudate was often found, while the lungs were congested and oedematous and sometimes showed petechia. No apparent lesion was seen on the heart.

1. — 4. Histological observations.

The sections observed all showed, to varying stages, microscopic lesions. It was observed that all the organs are the sites of congestive and hemorrhagic lesions. The results are summarized in Table 1.

TABLEAU 2 TABLE 2

Pourcentage de mortalité des rats à la 24<sup>e</sup> heure et au 10<sup>e</sup> jour d'intoxication  
 Percentage of mortality of rats (at 24 hours and 10 days)

Dose (mg/kg)	Voie P.O. Oral route				Voie I.P. intra-peritoneal route				Voie I.V. intravenous route	
	♂		♀		♂		♀		♂	
	24 h	10 J	24 h	10 J	24 h	10 J	24 h	10 J	24 h	10 J
2					0	0				
2,83					0	33	0	11		
4					0	44	0	22		
5,66					0	66	0	55	0	22
8	0	0	0	0	66	77	0	66	22	44
11,32	0	11	0	11	77	88	55	88	44	66
16	0	22	11	11			77	100	88	88
22,64	0	33	11	33						
32	33	55	22	66						
45,28	44	66	33	77						

1. — 5. Percentages of mortality.

These figures are shown in Table 2.

1. — 6. Lethal doses.

In Table 3 are shown the essential toxicological results.

1. — 7. Evolution of body weight of the animals.

Figure 1 shows the variation of weights of the male rats after administration, by the three routes used of the maximum tolerated dose (MTD) and the minimum lethal dose (MLD). The MTD for intravenous administration is not shown. The intraperitoneal route led to the most important disturbances of weight for the two doses considered. Nevertheless, for the MTD, the animals returned to their initial weight after three or four days.

2. *Acute toxicity in the mouse*

2. — 1. Symptomatology.

As in the rat, the symptoms do not varied as a function of the sex of the animal or the means of administration. Pain was manifested by torsions of the abdomen and hollowing of the flanks, followed by the neuromuscular and respiratory symptoms already

described. However the latter were less pronounced than in the rat and the chronology of the general symptomatology seemed to be delayed.

2. — 2. Survival time.

The variations observed were related to both the dose and the route of administration : 2h to 48h for intravenous injection, 3h to 5 days for intraperitoneal administration, and 4h to 3 days for gastric tubing.

2. — 3. Macroscopic lesions.

They were very similar to those already described in the rat : congested state of the digestive tract, the liver and the kidneys were observed as well as peritoneal and pleural exudates, and pulmonary oedema. Petechia were found in some mice with red hepatization of the lungs.

2. — 4. Histological observations.

The histological examination of mouse tissue showed results similar to the rat, except than the lesions were more severe. The results are summarized in Table 4.

2. — 5. Percentage of mortality.

At high doses the animals died within 24 hours, especially after intravenous or oral

TABLEAU 3 TABLE 3  
Paramètres toxicologiques chez le Rat  
Toxicological data in Rat

Paramètres toxicologiques Toxicological data	Voie P.O. Oral route		Voie i.P. intrapéritoneale route		Voie i.V. intraveineuse route	
	♂	♀	♂	♀	♂	♀
Probit = f (log. dose)	$Y = 3,14x - 0,32$	$Y = 4,04x - 0,83$	$Y = 3,56x - 2,64$	$Y = 4,33x - 1,72$	$Y = 4,72x - 0,58$	
LD 50 (10 days) mg/Kg	30,53	27,79	4,59	5,70	8,57	
5 % confidence interval	22,43 — 41,54	21,77 — 35,46	3,65 — 5,79	4,65 — 6,98	7,01 — 10,48	
Dose maxima tolérée (mg/Kg)	8	8	2			
Maximum tolerated dose						
Dose minima mortelle (mg/Kg)	11,32	11,32	2,83	2,83	5,66	
Minimum lethal dose						

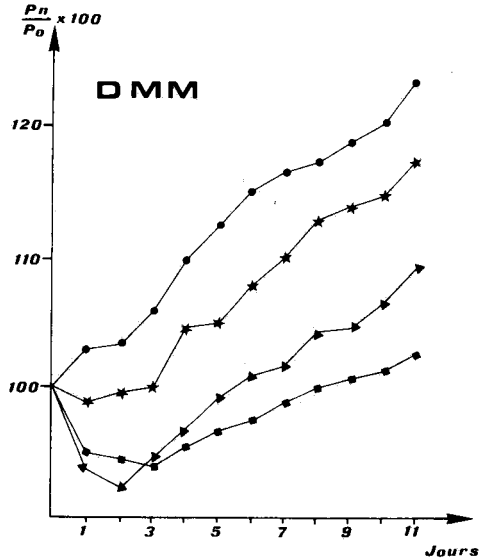
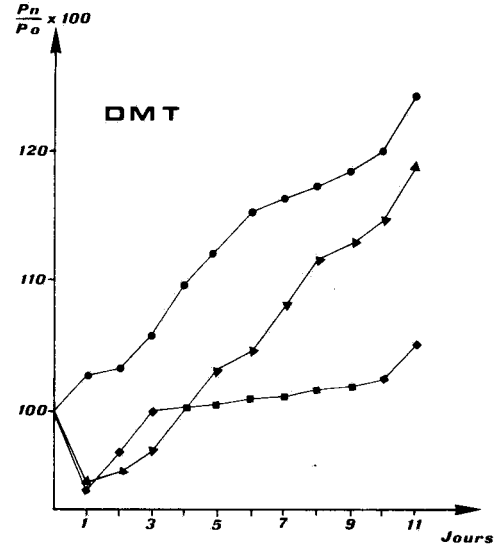


Fig. 1 : Bodyweight after treatment in rats :  
DMT = maximum non lethal dose.  
DMM = minimum lethal dose  
Controls (●), oral route (▲), intraperitoneal route (■), intravenous route (★).  
Po = weight at day 0 ; Pn = weight at day n.

TABLEAU 4    TABLE 4  
 Résultats des observations histologiques (Souris)  
*Histological results (Mice)*

Lésions	Rein <i>Kidney</i>	Foie <i>Liver</i>	Poumon <i>Lung</i>	Jéjunum <i>Jejunum</i>
Congestion <i>Hyperemia</i>	+++	+++	+++	+++
Hémorragies <i>Hemorrhages</i>	+++	+++	+++	—
Oedème <i>Oedema</i>	—	—	++	—
Anomalies nucléaires	<i>pycnose</i>	Hypochromie <i>pycnose</i>	<i>pycnose</i> intense	<i>pycnose</i> souvent généralisée à toute la coupe
<i>Nuclear abnormalities</i>	<i>pycnosis</i>	<i>Hypochromia Pycnosis</i>	<i>Intense pycnosis</i>	<i>Generalized pycnosis</i>
Nécrose <i>Necrosis</i>	++	—	++	+++
Ulcérations <i>Ulcers</i>	—	—	—	++
Divers <i>Micellaneous</i>	Intense dilatation des flocullus  <i>Intense dilatation of flocullus</i>	Intense infiltration lymphocytaire  <i>Intense leukocyte infiltration</i>	Infiltration lymphocytaire importante, atélectasie  <i>Leukocyte infiltration atelectasia</i>	Présence de polynucléaires éosinophiles dans la muqueuse  <i>Infiltration of mucosa by eosinophilic leukocytes</i>

Même légende que pour le tableau 1  
*Same legend as in table 1*

treatment; (Table 5) this phenomenon was identical in the rat.

## 2. — 6. Lethal doses.

The main toxicological parameters are shown in Table 6. As in the rat, the LD<sub>50</sub> values were significantly different between the oral and parenteral routes. A comparison of these results with those obtained for the rat showed that the mouse is less sen-

sitive, whatever the means of administration or the sex of the animal.

## 2. — 7. Evolution of body weight of the animals.

The evolution of the body weight of male mice after receiving the MTD and MLD is identical to that of the rats. The weight on the day treatment is regained after two or three days.

TABLEAU 5 TABLE 5

Pourcentages de mortalité des Souris (24 heures et 10 jours)

Percentages of mortality of mice (at 24 hours and 10 days)

Dose mg/Kg	Voie P.O. oral route				Voie I.P. intraperitoneal route				Voie I.V. intravenous route			
	♂		♀		♂		♀		♂		♀	
	24 h	10 J	24 h	10 J	24 h	10 J	24 h	10 J	24 h	10 J	24 h	10 J
2,83					0	0	0	0				
4					0	0	0	0				
5,66					0	22	0	22	0	0	0	0
8					11	55	33	66	0	0	0	0
11,32	0	0			11	66	55	77	0	22	0	11
16	0	0	0	0	100	100			11	44	33	44
22,64	0	11	0	0					33	66	44	77
32	33	33	0	11					66	88	77	88
45,28	55	66	44	55								
64	100	100	66	77								
90,56			100	100								

## Discussion

The symptomatology of acute patulin intoxication in the rat mouse was characterized mainly by signs of abdominal pain accompanied by locomotory and respiratory difficulties. Our observations do not reveal nervous system symptoms as previously described concerning alimentary intoxication of cattle, where patulin was indirectly implicated (Moreau and Moreau, 1960). Patulin intoxication remains to be verified, particularly in ruminants where the experimental chronic alimentary intoxication could eventually lead to a symptomatology different from that observed in the rat and mouse.

Macroscopic and microscopic lesions are not unequivocal. The congestive and hemorrhagic phenomena, in fact, remind the acute evolution of a non-specific process. Our observations enable us to consider on the one hand, an irritative and inflammatory mechanism following the action of patulin (infiltrations of acidophilic leukocytes, increase of Kupffer's cells, hyperplasia of lymphoid nodes ulcers and necrosis of the digestive tract) after oral administration, on the other hand, an eventual modification of hemodynamics could be considered (oedema, pleural and peritoneal exudates).

The exact mechanism of action of the toxin remains nevertheless unknown. Broom *et al.*

TABLEAU 6  
Paramètres toxicologiques chez la Souris  
Toxicological data in mice

Paramètre toxicologique Toxicological data	Voie P.O. oral route		Voie i.P. intraperitoneal route		Voie i.V. intravenous route	
	♂	♀	♂	♀	♂	♀
Prob <sub>50</sub> (log. dose)	Y = 7,07 x - 6,05	Y = 7,47 x - 7,44	Y = 6,25 x - 1,48	Y = 6,25 x - 1,48	Y = 4,96 x - 1,21	Y = 5,94 x - 2,43
LD 50 (10 days)	36,42	46,31	8,17	10,85	17,80	17,80
Int. confidence						
Conf. interval	30,90 — 42,93	39,50 — 54,28	6,84 — 9,76	9,02 — 13,05	14,50 — 21,85	14,84 — 21,36
5 %						
Dose maxima tolérée						
Maximum tolerated dose	16	22,64	4	4	8	8
mg/Kg						
Dose minima mortelle						
Minimum lethal dose	22,64	32	5,66	5,66	11,32	11, 32
mg/Kg						

(1944) reported a central action at the level of the spinal cord, the verification of which awaits a physiological exploration.

Whatever the route of administration, the mouse appears to be more resistant to patulin intoxication than the rat. This has already been noted for other mycotoxins, particularly for Aflatoxins (Legator, 1969) and for Ochratoxin A (Galtier *et al.*, 1974).

In the two animal species, a lesser toxicity observed after oral administration could be explained by :

- a delayed introduction of the toxin into the plasma,
- an elimination by the digestive tract,
- the activity of intestinal microbial flora.

The first explanation does not seem to be true for patulin. In fact, as opposed to the majority of toxins, its toxicity is weaker following venous introduction than following intraperitoneal administration. In the first case, the toxin diffuses directly and rapidly into the entire circulatory system. This phenomenon could perhaps be explained by the fixation of patulin on the sulfhydryl groups of glutathione at the level of red blood cell walls, (Andraud *et al.* 1969). According to Broom *et al.* (1944) and to Dulong de Rosnay *et al.* (1952) patulin would also be inactivated by rabbit and horse serum, as well as by human plasma and serum.

The possibility of an intestinal excretion of the mycotoxin can only be considered when we know the exact fate of the substance into the organism.

Finally, it is possible to suspect the action of intestinal flora ; Patulin is, in fact, a potent antibiotic whose mechanism of action is based on its fixation by means of a covalent bond to the SH groups of some bacterial enzymes (Geiger, 1945 ; Gottlieb and Singh, 1964).

Regarding the evolution of body weight of the animals, it is to be noted that the intoxicated subjects progressively regain their initial weight 2 to 4 days after treatment.

Although acute toxicity cannot entirely satisfy the toxicologist who deals with a natural contaminant. Nevertheless we believe that the numerical expression given by LD<sub>50</sub> enables the harmfulness character of a substance to be appreciated. In addition, we hope that the symptomatological conclusions can orient future biological research.

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