

Hoechst

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Hoechst Aktiengesellschaft
Pharma Research
Toxicology

REPORT

ON

AN ORAL MUTAGENICITY STUDY (MICRONUCLEUS TEST)

WITH OCTOPIROX IN MICE

Report No. 677/77

July 1, 1977

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Study of Octopirox for mutagenic activity in the
micronucleus test in NMRI mice after oral treatment

Summary:

Male and female NMRI mice received two oral treatments with doses of 125, 250 or 500 mg Octopirox per kg body weight in a 24-hour interval. Octopirox was suspended in starch mucilage. The control animals received the vehicle without drug. The animals were killed six hours after the second treatment. Bone marrow samples from the femur were applied onto microscopic slides, and then 2000 polychromatic erythrocytes and the proportional normochromatic erythrocytes per animal were counted and the number of polychromatic erythrocytes containing micronuclei were determined.

The studies showed that Octopirox did not lead to the formation of micronuclei in polychromatic erythrocytes in a mouse strain susceptible for the induction of micronuclei.

This result does not suggest a mutagenic activity of Octopirox.

Survey:

Compound: Octopirox (H 72 6146 A)
Operation E002

Vehicle 2 per cent starch mucilage

Type of study: Micronucleus test

Species: NMRI mouse

No. of animals per group: 10 male/10 female

Route: Oral, twice in a 24-hour interval

Dosage: 20 ml 2 per cent starch mucilage
per kg body weight (control)
125 mg Octopirox per kg body weight
250 mg Octopirox per kg body weight
500 mg Octopirox per kg body weight

Food: Altromin 1314 and tap water ad libitum

Maintenance: Five mice each on wood shavings in plastic cages

Room temperature: 20 - 22 °C

Relative humidity: 55 - 65 per cent

Killing: 6 hours after the second treatment

Kind of killing: Exarticulation of cervical vertebrae

Bone marrow preparation: Samples from femur applied onto microscopic slide, staining according to Wright

Evaluation: Number of micronuclei in 2000 polychromatic erythrocytes per animal

Duration of study: February 21 - March 4, 1977

Persons responsible:

Study director: Dr Baeder/Dr Horstmann

Statistics: Dr Passing

Industrial toxicology: Dr Weigand

Head of department: Prof. Kramer

Preliminary Remarks:

In this study, carried out in February/March 1977, Octopirox (H 72 6141 A) was examined for its mutagenic activity in a micronucleus test. The compound was taken from Batch E002.

The studies were performed in a mouse strain known for its susceptibility to the formation of micronuclei after oral treatment with 100 mg cyclophosphamide per kg body weight.

Method:

Groups of NMRI mice from our own breeding (strain HOE: NMRKf (SPF 71)), weighing 25 - 34 g (male animals) and 23 - 27 g (female animals) at the beginning of the study, received twice in a 24-hour interval orally by means of a stomach tube

1. 20 ml 2 per cent starch mucilage per kg body weight (control)
2. 125 mg Octopirox per kg body weight
3. 250 mg Octopirox per kg body weight
4. 500 mg Octopirox per kg body weight

The compound was suspended in 2 per cent starch mucilage and was administered to all animals in the same volume of 20 ml per kg body weight.

Each group consisted of 10 male and 10 female mice. The animals were marked individually and were kept under conventional conditions, separated according to sex, on wood shavings in plastic cages. The study was performed at a room temperature of 20 - 22 °C and a relative humidity of 55 - 65 per cent. The feed Altromin 1314 (mfr.: Altromin GmbH, Lage/Lippe; for composition see Appendix) and tap water were provided ad libitum.

The behaviour and the general state of health of the mice were assessed throughout the study.

Six hours after the second treatment the animals were killed by exarticulation of cervical vertebrae. The femur of each animal was cut off after removal of muscles and blood vessels. After removal of trochanter and condyles, the bone marrow was washed out into an Eppendorf vessel half-filled with fetal bovine serum*. The washing out was performed by means of an injection with fetal bovine serum. Subsequently the mixture was centrifuged for 5 minutes at 1000 rpm, the supernatant was decanted, and the sediment was suspended in the residual fluid by means of a Pasteur pipette. One drop of this cell suspension was smeared onto a microscopic slide, air-dried for approximately 24 hours and then stained in an Ames slide stainer according to Wright.

The smears of 5 males and 5 females per group were examined. Per animal, 2000 polychromatic erythrocytes and the proportional normochromatic erythrocytes were counted. The number of erythrocytes containing micronuclei was recorded. The quotient of normochromatic and polychromatic erythrocytes were statistically evaluated by means of the Wilcoxon test, and the frequency of occurrence of normochromatic and polychromatic erythrocytes with micronuclei was determined in a comparison of two binomial distributions. In both methods the results obtained in males and females each of the dosage groups were compared individually with those of the controls at a significance level of 97.5 per cent.

Results:

The administration of Octopirox did not affect the behaviour or impair the general state of health of any of the animals.

The findings obtained from the bone marrow smears are given in Tables 1 - 5. They show that the ratio of normochromatic to polychromatic erythrocytes was subject to marked individual variations in all dosage groups and control animals. Significant differences of this ratio between dosage groups and control animals were not observed. These findings did not suggest a depression in erythropoiesis.

The number of polychromatic erythrocytes containing micronuclei in all treated animals was within the range of previously obtained control values (0.8 - 2.6 per cent in males, 1.2 - 4.7 per cent in females). The number of normochromatic erythrocytes with micronuclei was within the same range. They represented the spontaneous rate in this study.

According to these results, in this micronucleus test performed in a mouse strain susceptible to micronucleus formation Octopirox did not show a mutagenic activity.

July 1, 1977

Pharmaceutical Research/Toxicology
of
HOECHST AG

signed:

Dr Baeder
Dr Horstmann
Dr Weigand
Prof. Kramer

* Fetal Bovine Serum for laboratory use, Flow Laboratories GmbH,
Bonn

Table 1

MICRONUCLEUS TEST

Survey of findings in bone marrow erythrocytes

Substance: OCTOPIROX

Route: oral

Animal: NMRI mouse

Sex	Dose	Number of animals	Erythrocytes polychr.			normochr.			Erythrocytes with micronuclei polychr.				normochr.			
			total No.	mean	2000	total No.	mean	% ¹	total No.	mean	%	mutagenic index ²	total No.	mean	%	mutagenic index ²
Male	Control	5	10000	2000		6516	1303	65	8	1.6	0.8	1.0	3	0.6	0.4	1.0
	125 mg/kg	5	10000	2000		6840	1368	68	7	1.4	0.7	0.8	8	1.6	1.1	2.6
	250 mg/kg	5	10000	2000		6985	1397	69	8	1.6	0.8	1.0	10	2.0	1.4	3.3
	500 mg/kg	5	10000	2000		8492	1698	84	13	2.6	1.3	1.6	13	2.6	1.5	4.3
Female	Control	5	10000	2000		7709	1541	77	9	1.8	0.9	1.0	10	2.0	1.2	1.0
	125 mg/kg	5	10000	2000		7704	1540	77	7	1.4	0.7	0.7	6	1.2	0.7	0.6
	250 mg/kg	5	10000	2000		9755	1951	97	13	2.6	1.3	1.4	7	1.4	0.7	0.7
	500 mg/kg	5	10000	2000		8889	1777	88	8	1.6	0.8	0.8	8	1.6	0.8	0.8

$$1 = \frac{\text{total No. of normochr. erythrocytes}}{\text{total No. of polychr. erythrocytes}} \times 100$$

$$2 = \frac{\text{erythrocytes with micronuclei in experimental group}}{\text{erythrocytes with micronuclei in control}}$$

Table 2

MICRONUCLEUS TEST
Findings in bone marrow erythrocytes

Substance: 2% starch mucilage
(control)

Dose: 20 ml/kg
Route: oral

Animal: NMRI mouse

Animal No.	Male				Animal No.	Female									
	Erythrocytes		Erythrocytes with micronuclei			Erythrocytes		Erythrocytes with micronuclei							
	polychr. No.	normochr. No.	%	polychr. No.	%	normochr. No.	%	polychr. No.	%	normochr. No.	%	polychr. No.	%	normochr. No.	%
201	2000	788	39	2	1.0	0	-	211	2000	1434	71	3	1.5	2	1.3
202	2000	1474	73	3	1.5	1	0.6	212	2000	1581	79	2	1.0	2	1.2
203	2000	1603	80	0	-	0	-	213	2000	967	48	1	0.5	0	-
204	2000	1317	65	2	1.0	2	1.5	214	2000	2768	138	2	1.0	4	1.4
205	2000	1334	66	1	0.5	0	-	215	2000	959	47	1	0.5	2	2.0
Total No.	10000	6516		8		3			10000	7709		9		10	
Mean	2000	1303		1.6		0.6			2000	1541		1.8		2.0	
%			65								77				
%					0.8		0.4						0.9		1.2
Mutagenic index****				1		1						1		1	

∫ = $\frac{\text{total No. of normochr. erythrocytes}}{\text{total No. of polychr. erythrocytes}} \times 100$

**** Mutagenic index = $\frac{\text{erythrocytes with micronuclei in experimental group}}{\text{erythrocytes with micronuclei in control}}$

Table 3

MICRONUCLEUS TEST
Findings in bone marrow erythrocytes

Substance: OCTOPIROX

Dose: 125 mg/kg

Animal: NRI mouse

Route: oral

Animal No.	M a l e				F e m a l e										
	Erythrocytes polychr. No.	normochr. No.	%	Erythrocytes with micronuclei polychr. No.	%	normochr. No.	%	Erythrocytes polychr. No.	normochr. No.	%	Erythrocytes with micronuclei polychr. No.	%	normochr. No.	%	
261	2000	1197	59	1	0.5	1	0.8	271	2000	785	39	4	2.0	2	2.5
262	2000	1403	70	2	1.0	3	2.1	272	2000	1320	66	2	1.0	0	-
263	2000	1817	90	1	0.5	2	1.1	273	2000	1379	68	1	0.5	2	1.4
264	2000	1181	59	0	-	0	-	274	2000	2092	104	0	-	0	-
265	2000	1242	62	3	1.5	2	1.6	275	2000	2128	106	0	-	2	0.9
Total No.	10000	6840		7		8			10000	7704		7		6	
Mean	2000	1368		1.4		1.6			2000	1540		1.4		1.2	
%			68								77				
%					0.7		1.1						0.7		0.7
Mutagenic index ^{***}				0.8		2.6						0.7		0.6	

$$* \quad \% = \frac{\text{total No. of normochr. erythrocytes}}{\text{total No. of polychr. erythrocytes}} \times 100$$

$$*** \quad \text{Mutagenic index} = \frac{\text{erythrocytes with micronuclei in experimental group}}{\text{erythrocytes with micronuclei in control}}$$

Table 4

MICRONUCLEUS TEST
Findings in bone marrow erythrocytes

Substance: OCTOPIROX

Dose: 250 mg/kg

Animal: NMRI mouse

Route: oral

Animal No.	M a l e				F e m a l e										
	Erythrocytes polychr. No.	normochr. No.	%	Erythrocytes with micronuclei polychr. No.	normochr. No.	%	Erythrocytes with micronuclei polychr. No.	normochr. No.	%						
241	2000	1155	57	0	-	1	0.8	251	2000	2675	133	4	2.0	4	1.4
242	2000	1265	63	1	0.5	2	1.5	252	2000	1940	97	1	0.5	0	-
243	2000	1426	71	2	1.0	1	0.7	253	2000	1609	80	3	1.5	1	0.6
244	2000	1371	68	0	-	0	-	254	2000	1822	91	4	2.0	1	0.5
245	2000	1768	88	5	2.5	6	3.3	255	2000	1709	85	1	0.5	1	0.5
Total No.	10000	6985		8		10			10000	9755		13		7	
Mean	2000	1397		1.6		2.0			2000	1951		2.6		1.4	
%			69								97				
%					0.8		1.4						1.3		0.7
Mutagenic index***				1		3.3						1.4		0.7	

** $\% = \frac{\text{total No. of normochr. erythrocytes}}{\text{total No. of polychr. erythrocytes}} \times 100$

*** Mutagenic index = $\frac{\text{erythrocytes with micronuclei in experimental group}}{\text{erythrocytes with micronuclei in control}}$

Table 5

MICRONUCLEUS TEST
Findings in bone marrow erythrocytes

Substance: OCTOPIROX

Dose: 500 mg/kg

Animal: NMRI mouse

Route: oral

Animal No.	Male				Female										
	Erythrocytes polychr. No.	normochr. No.	%	Erythrocytes with micronuclei polychr. No.	normochr. No.	%	Erythrocytes with micronuclei polychr. No.	normochr. No.	%						
221	2000	1377	68	4	2.0	4	2.9	231	2000	1119	55	2	1.0	3	2.6
222	2000	1732	86	1	0.5	2	1.1	232	2000	1294	64	1	0.5	2	1.5
223	2000	1293	64	3	1.5	1	0.7	233	2000	2192	109	2	1.0	2	0.9
224	2000	1725	86	2	1.0	3	1.7	234	2000	1925	96	1	0.5	1	0.5
225	2000	2365	118	3	1.5	3	1.2	235	2000	2359	117	2	1.0	0	-
Total No.	10000	8492		13		13			10000	8889		8		8	
Mean	2000	1698		2.6		2.6			2000	1777		1.6		1.6	
%			84								88				
to					1.3		1.5						0.8		0.8
Mitagenic index ^{****}				1.6		4.3						0.8		0.8	

$$* \quad \% = \frac{\text{total No. of normochr. erythrocytes}}{\text{total No. of polychr. erythrocytes}} \times 100$$

$$**** \quad \text{Mitagenic index} = \frac{\text{erythrocytes with micronuclei in experimental group}}{\text{erythrocytes with micronuclei in control}}$$

ALTROMIN Standard diets

Rats and Mice 1310

Bran 1311
4.5 mm Pellets 1313
10.0 mm Pellets 1314

TOTAL MICROBIAL COUNT

	Organisms/g - mean value (Mesentericus subtilis Gr.)
Normal and fortified diet	15 000
SPF diet	3 000

SUPPLEMENTARY CONTROLS

Antibiotic activity
Aflatoxins
Insecticides
Estrogens

CRUDE NUTRIENTS
(% - mean value)

Protein	23.5
Fat	6.0
Fiber	4.0
Ash	6.5
Water	12.5

Convertible energy
(Kcal/g)

3.2

MINERALS
(% - mean value)

Calcium	1.1
Phosphorus	0.9
Magnesium	0.2
Sodium	0.3
Potassium	0.7
Chlorine	0.8

AMINO ACIDS

(% crude protein - mean value)

Lysine	6.5
(lysine available)	(2.8)
Methionine + cysteine	4.3
Phenylalanine + tyrosine	8.0
Arginine	6.0
Histidine	2.5
Tryptophan	1.3
Threonine	4.1
Isoleucine	5.5
Leucine	8.1
Valine	5.4

TRACE ELEMENTS

(mg in 1000 g diet - mean value)

Manganese	85
Iron	185
Copper	15
Zinc	50
Iodine	1.5
Fluorine	0.4

VITAMINS in 1000 g diet (additives)

	Normal diet	SPF diet
Vitamin A	15 000 I.U.	25 000 I.U.
Vitamin D3	1 600 I.U.	2 800 I.U.
Vitamin E	75 mg	125 mg
Vitamin K3	3 mg	5 mg
Vitamin B1	18 mg	30 mg
Vitamin B2	12 mg	20 mg
Vitamin B6	9 mg	15 mg
Vitamin B12	24 µg	40 µg
Nicotinic acid	36 mg	60 mg
Pantothenic acid	21 mg	35 mg
Folic acid	2 mg	3 mg
Biotin	60 µg	100 µg
Choline	36 mg	60 mg
Vitamin C		