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REPORT OF THE BIOLOGICAL LABORATORY

Skin irritation study in
albino guinea pigs using
1-Methoxy-2-amino-4-(- β -hydroxy-ethylamino)-benzene-sulphate





Study director:

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Darmstadt, November 7th 1979

Abstract:

Test substance: 1-Methoxy-2-amino-4- β -hydroxy-ethylamino -
benzene-sulphate
1 percent suspended with gum Arabic (10 percent)

Object and method
of experiment: Determination of the skin compatibility of the test
substance through repeated dermal application to
albino guinea pigs.

Result: Under the given experimental conditions, the test
substances was well tolerated by the skin.

Beginning of
experiment: August 13th 1979

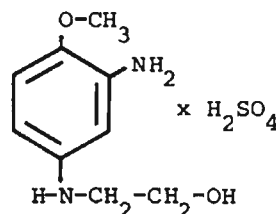
End of
experiment: August 17th 1979

Place of
experiment: Biological Laboratory
WELLA AG
Berliner Allee 65

6100 Darmstadt

I. Test substance

- a) 1-Methoxy-2-amino-4-β-hydroxy-ethylamino-benzene-sulphate,
percent suspended with gum Arabic (10 percent)



- b) Supplier: Bayer, Leverkusen
c) Nature: fine, grey powder

II. Application

The test substance was applied to an area of 3 x 4 cm of the flanks with a brush and was washed off after 20 minutes.

The treatment was repeated 3 times on two consecutive days.

III. Method

- A In analogy to the US-method according to Draize (2), the test substance was applied to areas of 3 x 4 cm of the clipped flanks*) of female albino guinea pigs.

B Experimental animals

For the test substance, 10 female albino guinea pigs of the SPF breed of Winkelmann*¹⁾ were used.

Body weight: 350 - 400 g

Age: 7 - 8 weeks

The average life expectancy is 6 - 7 years, the average tumour rate is 0,5 to 1,5 %.

Animal marking

All animals were ear-marked.

*) Electric clipper "Elektra II GH 204, 1/20 shears,
Messrs. Aeskulap

Substantiation of the choice of animals

Unlike the Draize-test, which suggests the use of rabbits, guinea pigs were used, because the Wella laboratory has been experimenting with the eyes of guinea pigs for many years.

C Housing

During the treatment period, the animals were kept immobilized in single cages.

During the subsequent observation period, the animals were kept in cages (of Ebeco²⁾) on standard "Bedding" of "Ssniff"³⁾.

The animals were given a standard laboratory diet (of Altromin⁴⁾) and water ad libitum.

Composition of standard diet:	see page 13
Composition of drinking water:	see page 12
Room temperature:	20°C ± 2°
Relative humidity:	50 % ± 5 % (maximum)
Air circulation:	approx. 15 times per hour
Alterating light/dark periods:	12 hours each
Light intensity:	approx. 350 lux.

D Application

The test substance was applied repeatedly to the right and left flanks of the animals on two consecutive days.

The treatment period was followed by a 3-day observation period.

E Evaluation

For assessing the skin reactions after each treatment and during the observation period, the following criteria were taken into account:

1. a) Erythema and eschar formation
- b) Edema formation

plus

2. Behaviour and general condition

F Determination of primary irritation index

The skin reactions were read by two persons independently and the mean values were calculated. The erythematous reactions observed were evaluated according to the following table (see Draize (4)):

a) Erythema and Eschar Formation

- | | | |
|-----|---|---|
| 0 | = | no erythema |
| 0,5 | = | very slight erythema (barely perceptible) |
| 1 | = | slight erythema |
| 2 | = | well-defined erythema |
| 3 | = | moderate to severe erythema |
| 4 | = | severe erythema (beet redness) to slight eschar formation (injuries in depth) |

Total possible erythema score = 4

b) Edema Formation

- 0 = no edema
- 1 = very slight edema (bare perceptible)
- 2 = slight edema (edges of area well defined by raising)
- 3 = moderate edema (raised approximately 1 mm)
- 4 = severe edema (raised more than 1 mm and extending beyond area of exposure)

Total possible edema score = 4

The primary irritation index was determined from the sum of the two average values of all erythema and edema scores.

The primary irritation index is the basis for the classification of the test substance:

- < 0,5 = non irritant
- 0,5 - 2,99 = mild irritant
- 3 - 4,99 = moderate irritant
- 5,99 = moderate to severe irritant
- 6 - 8 = severe irritant

IV. Evaluations:

1 a) Primary skin irritation

Under the given experimental conditions, two of ten animals showed a very slight erythema of the clipped not scarified skin.

1 b) There were no edemas observed.

On the last day of the experiment, all of the animals were free from symptoms.

Primary irritation index: $\bar{x} = 0,03$

Thus, the test substance was classified as "non irritant".

Detailed findings are given in table 1; for key to table 1 see page 5.

2 General behaviour of the animals

The preparation applied as described, did not produce any changes of the behaviour of the animals during the entire observation period.

V. Result

The test substance was well tolerated by the skin.

Reading after application	Animal No. 6		Animal No. 7		Animal No. 8		Animal No. 9		Animal No. 10		Mean reactions (\bar{x})
	left	right	left	right	left	right	left	right	left	right	
1st day 1st treatment		0		0		0		0		0	
2nd treatment		0		0		0		0		0	
3rd treatment		0		0		0		0		0	
2nd day 1st treatment		0,5		0		0		0		0	0,1
2nd treatment		0,5		0		0		0		0	0,1
3rd treatment		0,5		0		0		0		0	0,1
3rd day		0		0		0		0		0	
4th day		0		0		0		0		0	
5th day		0		0		0		0		0	
											\bar{x} : 0,03
											s: 0,045
											n: 10
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	
	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	
	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	
	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	Erythema	

REFERENCES

- (1) L. Cavalli-Sforza, Biometrie,
Grundzüge biologisch-medizinischer
Statistik, Gustav Fischer Verlag
Stuttgart, 1974, Seite 48 und 72

- (2) Hazardous Substances, Part 191, Section II,
FDA, Washington 1965

- (3) Zufallszahlen - entnommen aus L. Sachs
Angewandte Statistik, Springer Verlag
Berlin, Heidelberg, New York 1973,
Seite 44, Tabelle 10

- (4) Draize, Appraisal of the safety of
chemicals in food, drugs and cosmetics.
Editorial Office of USA 1959, 46 - 59

SUPPLIERS

*1. Winkelmann
Versuchstierzucht GmbH & Co. KG
Gartenstraße 300

4791 Borcheln 1

*2. Ebeco
E. Becker & Co. GmbH
Postfach 5 46

4620 Castrop-Rauxel

*3. Ssniff-Versuchstier-Diäten GmbH
Thomätor 3

4770 Soest

*4. Altromin GmbH
Tier-Labor-Service
Lange Straße 40

4937 Lage

W a t e r

Chemical analysis

Type of water: Drinking water
Place of sampling: Darmstadt, Frankfurter Str. 100
Time of sampling: January-June 1979 (mean values)
Appearance: colourless clear Odour: inodourous
Total hardness: 16.1 °d.H. Carbonate hardness: 11.3 °d.H*
Consumption of potassium permanganate: 1.4 mg KMnO₄/litre
m-Value: 4.0 pH-Value: 7.3

1 litre of water contains ... mg of

<u>Residue on evaporation (110°): --</u>	<u>Carbonic acid (CO₂):</u>
<u>Residue on ignition: --</u>	<u>Free CO₂: --</u>
<u>Calcium oxide (CaO): --</u>	<u>Combined CO₂: --</u>
<u>Calcium hardness: 12.2 °d.H</u>	
<u>Magnesium oxide (MgO):</u>	<u>Aggressive CO₂: --</u>
<u>Magnesium hardness: 3.9 °d.H.</u>	<u>Aggressive CO₂ (Heyer): 0</u>
<u>Total iron content (Fe): < 0.05</u>	<u>Nitrates (NO₃'): 13.0</u>
<u>Iron, bivalent (Fe^{''}): --</u>	<u>Nitrites (NO₂'): < 0.005</u>
<u>Iron, trivalent (Fe^{'''}): --</u>	<u>Sulphates (SO₄'): 55</u>
<u>Mangane (Mn): < 0.03</u>	<u>Phosphates (P₂O₅): < 0.2</u>
<u>Aluminium (Al): --</u>	<u>Sulphides (S'): --</u>
<u>Ammonia (NH₃): < 0.05</u>	<u>Hydrogen sulfide (H₂S): --</u>
<u>Chlorides (Cl'): 25'</u>	<u>t₀ = 10.6°</u>
<u>Fluorides (F'): --</u>	
<u>Silicic acid (SiO₂): 9.0</u>	
<u>Oxygen (O₂): 6.5</u>	

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SÜDHESSISCHE GAS UND WASSER AG, DARMSTADT
Laboratoriums-Leitung

* .1 degree of German hardness = 1.25 English hardness

altromin

Standard-diet
for Guinea-pigs

No.3020

Consistency:	flour	compact,	diameter (mm)		
		3	4.5	10	15
Order number:	3021	3022	-	-	3025

Special diet:
 - Upon demand
 - Suitable for SPF, no pre-treatment required
 - In heat-sealed plastic bags.

Crude nutrients

Crude protein	18.0
Crude fat	4.3
Crude fibre	13.5
Ash	8.9
Water	12.0
Nitrogen-free extracts	43.3
Convertible energy:	
Kcal/kg	2.700
KJ /kg	11.300

Amino acids

Lysine	0.9
Methionine + Cystine	0.5
Phenylalanin + Tyrosine	1.3
Arginine	1.0
Histidine	0.4
Tryptophane	0.2
Threonine	0.7
Isoleucine	0.9
Leucine	1.4
Valine	0.9

Minerals

Calcium	1.0
Phosphorous	0.7
Magnesium	0.2
Sodium	0.2

Tracer elements

Manganese	62.0
Iron	165.0
Copper	16.0
Zinc	50.0
Jodine	0.9
Fluorine	10.0

Vitamins

Standard-diet

Vitamin A	15.000 IE
Vitamin D ₃	600 IE
Vitamin E	75 mg
Vitamin K ₃	3 mg
Vitamin B ₁	18 mg
Vitamin B ₂	12 mg
Vitamin B ₆	9 mg
Vitamin B ₁₂	24 mcg
Nicotinic acid	36 mg
Pantothenic acid	21 mg
Folic acid	2 mg
Biotin	60 mcg
Choline	600 mg
Vitamin C	1.036 mg

Vitamins

Standard-diet (fortified) and special diet

Vitamin A	25.000 IE
Vitamin D ₃	1.000 IE
Vitamin E	125 mg
Vitamin K ₃	5 mg
Vitamin B ₁	30 mg
Vitamin B ₂	20 mg
Vitamin B ₆	15 mg
Vitamin B ₁₂	40 mcg
Nicotinic acid	60 mg
Pantothenic acid	35 mg
Folic acid	3 mg
Biotin	100 mcg
Choline	1.000 mg
Vitamin C	1.060 mg