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## REPORT OF THE BIOLOGICAL LABORATORY

### Acute Oral Toxicity Test of 1-Methoxy-2-amino-4- $\beta$ -hydroxyethylaminobenzene-sulphate





- 1 -

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Darmstadt, September 4th 1979

<u>Contents</u>	<u>page</u>
I. Summary	3
II. Method	
1. Test substance and application	4
2. Experimental animals	4
3. Housing	5
4. Toxicity criteria	6
III. Results	6
a) Groups and dosage levels	6
b) LD <sub>50</sub>	7
c) Mortalities	7 and
d) Body weights	7 and 9 - 19
e) Observations and post-mortem findings	7
f) Clinico-toxicological observations	22 and 23
IV. Evaluation	8
V. Tables	9-22
VI. References	23
VII. Suppliers	24
VIII. Composition of standard diet	25
IX. Composition of drinking water	26

**Summary:** Under the given experimental conditions, the following results were obtained:

1. The test substance, administered in the doses indicated, caused toxicity symptoms: Tonic spasm, piloerection, higher respiration rate and exitus.
2. The LD<sub>50</sub>:  
588 mg/kg body weight female rat, Wistar breed  
475 mg/kg body weight male rat, Wistar breed  
538 mg/kg body weight female mice, CF 1 breed
3. According to Hodge and Sterner, the test substance can be classified as "moderately toxic to slightly toxic".

**Test substance:** 1-Methoxy-2-amino-4-β-hydroxyethylamino-benzene-sulphate.  
Colipa No.: A 84, Colour Index: %.

**Object and method of experiment:** It was the objective of the experiment to establish the toxicity of the test substance upon single oral administration in rats and mice. The median lethal dose was determined during a 2-week observation period.

**Beginning of experiment:** July 25<sup>th</sup> 1979

**End of experiment:** August 28<sup>th</sup> 1979

II. Method

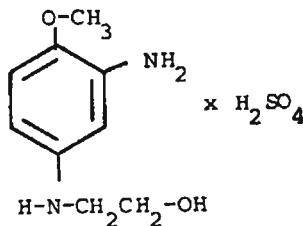
The experiments were carried out in analogy to "Appraisal of the safety of chemicals in foods, drugs and cosmetics" (Food and Drug Administration)

All mortalities and signs of toxicity were recorded. Each animal was examined for organ lesions.

The LD<sub>50</sub> was determined from the observations made and subsequently the test substance was classified according to Hodge and Sterner (Amer. Indust. Hyg. Ass. 10, 93-96, 1949).

1. Test substance and application

1-Methoxy-2-amino-4-β-hydroxyethylaminobenzene-sulphate,  
fine, grey powder



The test substance was administered by stomach tube, 5 and 10 percent aqueous solution.

2. Experimental animals

30 Female rats, Wistar breed

30 Male rats, Wistar breed

70 Female mice, CF 1 breed

SPF breeds of Winkelmann<sup>\*1</sup>

The rats were chosen because their metabolism is very similar to that of man. In order not to miss any differences of the effect of the test substance, mice were also used for the experiment.

The animals were randomized with the aid of an appropriate table (1).

The animals were marked with picric acid according to Teuffel (2), and the cages were provided with identification tags.

### 3. Housing

The animals were off food for 16 hours before treatment, because in oral toxicity tests the stomach contents are of great importance, and in order to make starting conditions as uniform as possible. Two hours after treatment, the animals were again offered food. The animals were housed in suspended wire cages.

During the two-week observation period, the animals were kept in groups, 5 female rats and 10 female mice each, in Macrolon cages, type III, of Ebeco<sup>#2</sup> on standard "Bedding" of Ssniff<sup>#3</sup>.

The animals were given a standard laboratory diet of Altromin<sup>#4</sup> and water ad libitum.

Composition of standard laboratory diet: See page 25

Composition of drinking water: See page 26

The room temperature was  $20^{\circ} \text{C} \pm 2^{\circ} \text{C}$ , the relative humidity  $50 \% \pm 5 \%$  (maximum). Circulation of air: approximately 15 times/hour. There were alternating light and dark periods of 12 hours each.

Intensity of light: approx. 390 lux.

4. Toxicity criteria in main experiment

Mortalities	-	daily (table 1 and 2)
Body weights	-	weekly (table 1)
Clinico-toxicological observations	-	daily
Dissection	-	all animals were dissected (table 1)

III. Results

a) Groups and dose levels

In a preliminary experiment, the approximate LD<sub>50</sub> value was determined (range finding):

Dose level g/kg body weight	number of animals died			
	Mortality = $\frac{\text{number of animals died}}{\text{number of animals treated}}$			
	rats ♀	rats ♂	mice ♀	time
1,500	-	-	10/10	> 24 <sup>h</sup>
0,250	-	-	0/10	-
0,875	5/5	5/5	10/10	> 24 <sup>h</sup>

The preliminary experiment on the mice showed that the median lethal dose is lower than 0,875 g/kg body weight.

In the main experiment on the mice and rats, corresponding dose levels were tested in a larger number of animals.

b) Table 1 shows the individual results. The LD<sub>50</sub> was calculated on the basis of the Spearman-Kärber method (3) with 95 percent confidence.

	rat ♀	rat ♂	mouse ♀
LD <sub>50</sub> mg/kg	588	475	538
lower level	311	265	187
upper level	864	685	886

c) Mortalities

See table 2

d) Body weights

There was a continuous increase in body weight in all surviving animals over the entire period (see table 1a - 1k)

e) Observations and post-mortem findings

There were no pathological changes of the organs of the abdominal and thoracic cavities and of the central nervous system (see table 1a - 1k).



IV. Evaluation

Based on the observations made, the tested substance can be classified according to Hodge and Sterner as "moderately toxic" by f.rats and "slightly toxic" by m.rats and f.mice.

Table 1a

## Acute Toxicity - main experiment

Dose: 0,250g/kg  
Species: mouse - CF 1

( 5 percent aqueous solution )

8/14/79

Animal No.	Sex	Body weight (g)	Appl. Vol. (ml)	Exitus after	Body weight after		Post-mortem findings
					7 days	14 days	
1	♀	29	0.15	-	36	33	-
2	♀	28	0.14	-	31	31	-
3	♀	28	0.14	-	32	32	-
4	♀	28	0.14	-	33	31	-
5	♀	23	0.12	-	23	34	-
6	♀	23	0.12	-	25	27	-
7	♀	25	0.13	-	28	27	-
8	♀	30	0.15	-	28	33	-
9	♀	31	0.16	-	32	34	-
10	♀	24	0.12	-	26	28	-

Table 1b

## Acute Toxicity - main experiment

Dose: 0.375 g/kg  
Species: mouse - CF 1

8/14/79

Animal No.	Sex	body weight (g)	Appl. Vol. (ml)	Exitus after	Body weight after		Post-mortem findings
					7 days	14 days	
1	♀	31	0.23	-	32	34	-
2	♀	26	0.2	-	28	27	-
3	♀	26	0.2	< 24 <sup>h</sup>	-	-	-
4	♀	29	0.22	-	33	29	-
5	♀	23	0.17	-	28	26	-
6	♀	24	0.18	-	26	28	-
7	♀	26	0.2	-	29	32	-
8	♀	29	0.22	-	28	32	-
9	♀	26	0.2	< 24 <sup>h</sup>	-	-	-
10	♀	26	0.2	-	30	31	-



Table 1a

## Acute Toxicity - main experiment

Dose: 0,625 g/kg

Species: mouse - CF 1

7/25/79

Animal No.	Sex	Body weight (g)	Appl. Vol. (ml)	Exitus after	Body weight after		Post-mortem findings
					7 days	14 days	
1	♀	27	0.17	-	30	32	
2	♀	24	0.15	-	27	28	
3	♀	25	0.16	<24 <sup>h</sup>	-	-	No abnormality discovered
4	♀	26	0.16	-	29	33	
5	♀	27	0.17	48 <sup>h</sup>	-	-	
6	♀	25.	0.16	-	31	35	
7	♀	25	0.16	<24 <sup>h</sup>	-	-	
8	♀	27	0.17	<24 <sup>h</sup>	-	-	
9	♀	23	0.14	-	27	30	
10	♀	29	0.18	<24 <sup>h</sup>	-	-	

Dose: 0,75 g/kg  
 Species: mouse - CF 1

7/25/79

Acute Toxicity - main experiment

Table 1e

Animal No.	Sex	Body weight (g)	Appl. Vol. (ml)	Exitus after	Body weight after		Post-mortem findings
					7 days	14 days	
1	♀	22	0.17	<24 <sup>h</sup>	-	-	
2	♀	27	0.2	<24 <sup>h</sup>	-	-	
3	♀	25	0.19	-	27	30	
4	♀	24	0.18	<24 <sup>h</sup>	-	-	
5	♀	21	0.16	<24 <sup>h</sup>	-	-	
6	♀	26	0.2	<24 <sup>h</sup>	-	-	
7	♀	21	0.16	<24 <sup>h</sup>	-	-	
8	♀	27	0.2	<24 <sup>h</sup>	-	-	
9	♀	25	0.19	<24 <sup>h</sup>	-	-	
10	♀	24	0.18	<24 <sup>h</sup>	-	-	

NO ABNORMALITY DISCOVERED

Table 1 f

Acute Toxicity - main experiment

Dose: 0,875 g/kg  
 Species: mouse - CF 1

7/25/79

Animal No.	Sex	Body weight (g)	Appl. Vol. (ml)	Exitus after	Body weight after		Post-mortem findings
					7 days	14 days	
1	♀	22	0.19	<24 <sup>h</sup>	-	-	
2	♀	26	0.23	<24 <sup>h</sup>	-	-	
3	♀	23	0.2	<24 <sup>h</sup>	-	-	
4	♀	26	0.23	<24 <sup>h</sup>	-	-	
5	♀	27	0.24	<24 <sup>h</sup>	-	-	
6	♀	29	0.25	<24 <sup>h</sup>	-	-	
7	♀	22	0.19	<24 <sup>h</sup>	-	-	
8	♀	25	0.22	<24 <sup>h</sup>	-	-	
9	♀	24	0.21	<24 <sup>h</sup>	-	-	
10	♀	25	0.22	<24 <sup>h</sup>	-	-	

NO ABNORMALITY DISCOVERED

Table 19

## Acute Toxicity - main experiment

Dose: 0,25 g/kg

Species: rat - Wistar

8/14/79

Animal No.	Sex	Body weight (g)	Appl. Vol. (ml)	Exitus after	Body weight after		Post-mortem findings
					7 days	14 days	
1	♀	180	0.45	-	191	201	-
2	♀	192	0.48	-	196	216	-
3	♀	193	0.48	-	208	209	-
4	♀	191	0.48	-	200	202	-
5	♀	178	0.45	-	191	196	-
1	♂	310	0.78	-	289	321	-
2	♂	292	0.73	-	302	317	-
3	♂	298	0.75	-	293	315	-
4	♂	310	0.78	-	308	325	-
5	♂	253	0.63	-	241	289	-





Dose: 0,5 g/kg  
 Species: rat - Wistar  
 8/14/79

Acute Toxicity - main experiment

Table 1 i

Animal No.	Sex	Body weight (g)	Appl. Vol. (ml)	Exitus after	Body weight after		Post-mortem findings
					7 days	14 days	
1	♀	179	0.89	-	189	192	-
2	♀	186	0.93	5 days	-	-	-
3	♀	197	0.99	-	212	212	-
4	♀	192	0.96	4 days	-	-	-
5	♀	204	1.02	-	209	210	-
1	♂	334	1.67	-	322	350	-
2	♂	271	1.36	4 days	-	-	-
3	♂	286	1.43	4 days	-	-	-
4	♂	265	1.33	5 days	-	-	-
5	♂	296	1.48	2 days	-	-	-

Table 1 j

## Acute Toxicity - main experiment

Dose: 0.625 g/kg  
Species: rat - Wistar

8/15/97

Animal No.	Sex	Body weight (g)	Appl. Vol. (ml)	Exitus after	Body weight after		Post-mortem findings
					7 days	14 days	
1	♀	150	0.94	< 24 <sup>h</sup>	-	-	-
2	♀	135	0.84	2 days	-	-	-
3	♀	194	1.21	4 days	-	-	-
4	♀	134	0.84	-	143	161	-
5	♀	141	0.88	5 days	-	-	-
1	♂	282	1.76	3 days	-	-	-
2	♂	299	1.87	5 days	-	-	-
3	♂	293	1.83	4 days	-	-	-
4	♂	290	1.81	3 days	-	-	-
5	♂	274	1.71	4 days	-	-	-



Table 2 - Mortalities in preliminary and main experiment

Animal	Animal No.	Dosage g/kg body weight	Mortalities
♀ Mouse CF <sub>1</sub>	10	0.250	0/10
	10	0.375	2/10
	10	0.500	6/10
	10	0.625	5/10
	10	0.750	9/10
	10	0.875	10/10
	10	1.500	10/10
♀ Rat-Wistar	5	0.250	0/5
	5	0.375	2/5
	5	0.500	2/5
	5	0.625	4/5
	5	0.750	5/5
	5	0.875	5/5
♂ Rat-Wistar	5	0.250	0/5
	5	0.375	3/5
	5	0.500	4/5
	5	0.625	5/5
	5	0.750	5/5
	5	0.875	5/5

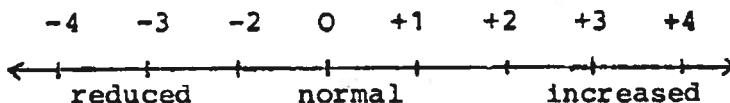
Table 3 Clinico-toxicological observations

Experiment: Acute oral Toxicity

Evaluation:

Dosage: 0,250 g/kg - 0,750 g kg

Species: ♀ and ♂ rats



	Observations	1 <sup>h</sup>	2 <sup>h</sup>	24 <sup>h</sup>	2nd-5th day	7th day	14th day
Emo- Con- scious- ness	Activity						
	General reaction						
	Pain reaction						
	Articulation						
	Unrest						
	Excitability/aggressiveness						
Central symptoms	Stereotypy						
	Tremor						
	Tonic spasms	+1	+2	0			
	Clonic spasms						
Coordi- nation	Reeling						
	Ataxies						
	Lateral position						
	Righting reflex (0-8)						
	Abnormalities in posture						
Toni- city	Tonus extremities						
	Body tonus				EX	N	N
	Tension of abdominal wall				IT	OR	OR
	Cutaneous turgor				U	R	R
Re- fle- xes	Ear reflex				S	N	N
	Plantar reflex				U	R	R
	Corneal reflex				S	N	N
Autonomous functions	Size of the pupils					*	
	Exophthalmus						
	Complexion (skin and mucosa)						
	Diuresis/diarrhea						
	Salivation						
	Pilo-erection		+1	0			
	Respiratory rate		+1	0			
	Body temperature						

\* normal at the living animals

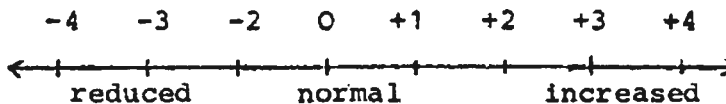
Table 3 Clinico-toxicological observations

Experiment: Acute oral Toxicity

Evaluation:

Dosage: 0.250 g/kg - 0.875 g/kg

Species: Mouse CF<sub>1</sub>



	Observations	1 <sup>h</sup>	4 <sup>h</sup>	24 <sup>h</sup>	48 <sup>h</sup>	7th day	14th day
Consciousness	Activity						
	General reaction						
	Pain reaction						
Emotion	Articulation						
	Unrest						
	Excitability/aggressiveness						
Central symptoms	Stereotypy						
	Tremor						
	Tonic spasms	+ 1	+ 3				
	Clonic spasms						
Coordination	Reeling						
	Ataxies						
	Lateral position						
	Righting reflex (0-8)						
	Abnormalities in posture						
Tonicity	Tonus extremities						
	Body tonus				EXCITUS	NORMAL	NORMAL
	Tension of abdominal wall				EXCITUS	NORMAL	NORMAL
Reflexes	Cutaneous turgor				EXCITUS	NORMAL	NORMAL
	Ear reflex						
	Plantar reflex						
Autonomous functions	Corneal reflex						
	Size of the pupils						
	Exophthalmus						
	Complexion (skin and mucosa)						
	Diuresis/diarrhea						
	Salivation						
	Pilo-erection	+1	+1				
	Respiratory rate	+ 1	+ 1				
Body temperature							

\* normal at the living animals

References

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Angewandte Statistik, Springer-Verlag Berlin.  
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- (2) S. Jung, Grundlagen für die Zucht und Haltung der  
wichtigsten Versuchstiere, Gustav Fischer Verlag,  
Stuttgart 1962, Seite 242
  
- (3) Spearman-Kärber Methode - siehe L. Sachs,  
Angewandte Statistik, Springer Verlag  
Berlin, Heidelberg, New York, 1973  
Seite 17<sup>A</sup>



SUPPLIERS

- \*1. Winkelmann  
Versuchstierzucht GmbH & Co. KG  
Gartenstraße 300  
  
4791 Borchten 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

altromin

Standard-diet  
for Guinea-pigs

No.3020

Consistency:	flour	compact, diameter (mm)			
		3	4.5	10	15
Order number:	3021	3022	-	-	3025
<u>Special diet:</u>	- Upon demand - Suitable for SPF, no pre-treatment required - In heat-sealed plastic bags.				
<u>Crude nutrients</u>		<u>Amino acids</u>			
Crude protein	18.0	Lysine	0.9		
Crude fat	4.3	Methionine + Cystine	0.5		
Crude fibre	13.5	Phenylalanin + Tyrosine	1.3		
Ash	8.9	Arginine	1.0		
Water	12.0	Histidine	0.4		
Nitrogen-free		Tryptophane	0.2		
extracts	43.3	Threonine	0.7		
Convertible energy:		Isoleucine	0.9		
Kcal/kg	2.700	Leucine	1.4		
KJ /kg	11.300	Valine	0.9		
<u>Minerals</u>		<u>Tracer elements</u>			
Calcium	1.0	Mangane	62.0		
Phosphorous	0.7	Iron	165.0		
Magnesium	0.2	Copper	16.0		
Sodium	0.2	Zinc	50.0		
		Jodine	0.9		
		Fluorine	10.0		
<u>Vitamins</u>		<u>Vitamins</u>			
<u>Standard-diet</u>		<u>Standard-diet (fortified) and special diet</u>			
Vitamin A	15.000 IE	Vitamin A	25.000 IE		
Vitamin D <sub>3</sub>	600 IE	Vitamin D <sub>3</sub>	1.000 IE		
Vitamin E	75 mg	Vitamin E	125 mg		
Vitamin K <sub>3</sub>	3 mg	Vitamin K <sub>3</sub>	5 mg		
Vitamin B <sub>1</sub>	18 mg	Vitamin B <sub>1</sub>	30 mg		
Vitamin B <sub>2</sub>	12 mg	Vitamin B <sub>2</sub>	20 mg		
Vitamin B <sub>6</sub>	9 mg	Vitamin B <sub>6</sub>	15 mg		
Vitamin B <sub>12</sub>	24 mcg	Vitamin B <sub>12</sub>	40 mcg		
Nicotinic acid	36 mg	Nicotinic acid	60 mg		
Pantothenic acid	21 mg	Pantothenic acid	35 mg		
Folic acid	2 mg	Folic acid	3 mg		
Biotin	60 mcg	Biotin	100 mcg		
Choline	600 mg	Choline	1.000 mg		
Vitamin C	1.036 mg	Vitamin C	1.060 mg		

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 KMnO<sub>4</sub>/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 (CO<sub>2</sub>):</u>
<u>Residue on ignition: --</u>	<u>Free CO<sub>2</sub>: --</u>
<u>Calcium oxide (CaO): --</u>	<u>Combined CO<sub>2</sub>: --</u>
<u>Calcium hardness: 12.2 °d.H</u>	
<u>Magnesium oxide (MgO):</u>	<u>Aggressive CO<sub>2</sub>: --</u>
<u>Magnesium hardness: 3.9 °d.H.</u>	<u>Aggressive CO<sub>2</sub> (Heyer): 0</u>
<u>Total iron content (Fe): &lt; 0.05</u>	<u>Nitrates (NO<sub>3</sub>' ): 13.0</u>
<u>Iron, bivalent (Fe''): --</u>	<u>Nitrites (NO<sub>2</sub>' ): &lt; 0.005</u>
<u>Iron, trivalent (Fe'''): --</u>	<u>Sulphates (SO<sub>4</sub>' ): 55</u>
<u>Manganese (Mn): &lt; 0.03</u>	<u>Phosphates (P<sub>2</sub>O<sub>5</sub>): &lt; 0.2</u>
<u>Aluminium (Al): --</u>	<u>Sulphides (S' ): --</u>
<u>Ammonia (NH<sub>3</sub>): &lt; 0.05</u>	<u>Hydrogen sulfide (H<sub>2</sub>S): --</u>
<u>Chlorides (Cl' ): 25'</u>	<u>t<sub>0</sub> = 10.6°</u>
<u>Fluorides (F' ): --</u>	
<u>Silicic acid (SiO<sub>2</sub>): 9.0</u>	
<u>Oxygen (O<sub>2</sub>): 6.5</u>	

SÜDHESSISCHE GAS UND WASSER AG, DARMSTADT  
Laboratoriums-Leitung

\* 1 degree of German hardness = 1.25 English hardness