

***Chemo-prevention of the toxicity of nickel, and of the mutagenic toxicities of cobalt, urethane and formaldehyde in Drosophila melanogaster by Avemar***

**1. Toxicity examination**

In the experiments we examined whether the fermented wheat germ extract, Avemar influences the toxicity of nickel-chloride. We mixed nickel-chloride – in various concentrations - into standard nutrient containing 10% Avemar and fed animals taken from 10-day Oregon species. Every group featured 10 males and 10 females. As control we mixed the nickel-chloride into a nutrient containing 10% sacharose. The treatment and the observation took 13 days at 25°C. The hatching of Drosophila takes place on the 10<sup>th</sup> day from laying the egg, therefore on the 13<sup>th</sup> day we determined the total number of animals still alive and hatched anew. The toxicity level was the decreasing number of the dying and hatching flies. The results are presented in Table 1.

Table 1. The Avemar effect on the nickel-chloride toxicity

<b>Number of days →</b>	<b>1</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>13</b>
Treatment	<b>number of deceased</b>						<b>survivors and descendants</b>
12 mM NiCl <sub>2</sub> + 10% Avemar	0	3	12	14	19	20	0
12 mM NiCl <sub>2</sub> + 10% sacharose*	0	9	13	19	20	20	0
6 mM NiCl <sub>2</sub> + 10% Avemar	0	0	0	0	0	0	85
6 mM NiCl <sub>2</sub> + 10% sacharose	0	4	4	5	10	10	0
3 mM NiCl <sub>2</sub> + 10% Avemar	0	0	0	0	0	0	131
3 mM NiCl <sub>2</sub> + 10% sacharose	0	0	0	0	0	0	18
10% Avemar	0	0	0	0	0	0	116
10% sacharose	0	0	0	0	0	0	19

\* We added the 10% sacharose to the finished standard nutrient (already containing sacharose) subsequently, as an addendum.

Evaluation

Avermar had shown a strong protective effect in *Drosophila* when the animals were treated with 6 mM nickel-chloride. Here – without Avermar - all the animals died by the 13<sup>th</sup> day, while protected with Avermar there were descendants, too. On the 13<sup>th</sup> day there was a significant difference in the number of the live adult animals treated with 3 mM nickel-chloride for those treated with Avermar. This was also due to the fact – besides the protective one - that the Avermar quickens the development of the flies. This is visible in the two groups being not treated with nickel where on the 13<sup>th</sup> day the total number of the survivors and descendants on nutrient containing 10% Avermar was 116 while this number was 19 on the nutrient containing 10% sacharose as addendum.

## 2. Examining the mutagenity

In the tests we checked whether Avermar influences the mutagenity of cobalt-chloride, urethane and formaldehyde in *Drosophila*.

### Method

The level of the mutagene effect was evaluated with the *Drosophila* somatic mutation and recombination test. We treated larvae that carry one-one recessive mutations on their third chromosome (*mwh/flr*). In case of a new mutation or somatic recombination taking place at the proper location the mutant genes get into homo-zygote status, and get visible on the body of the adult animals. This is evaluated on the wings with a light microscope at a magnification of 400.

We mixed the examined mutagene solutions on their own or with Avermar in a proper concentration described in the literature into a melted *Drosophila*-nutrient. We placed the 72+-4 hours larvae on this after cooling. Until hatching the animals were kept in a thermostat of 25 degrees.

### Results

The results are presented in tables 2., 3. and 4..

Table 2. Effect of Avermar onto the mutagenity of cobalt-chloride

Treatment	Mutation/wing	Mutational frequency	Significance Kastenbaum-B.
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			<b>test</b>
2 mM CoCl <sub>2</sub> + 10% sacharose*	77/32	2,41	
2 mM CoCl <sub>2</sub> + 10% Avemar	24/32	0,75	p<0,01
10% sacharose	33/40	0,83	
10% Avemar	28/40	0,70	

\* We added We added the 10% sacharose to the finished standard nutrient (already containing sacharose) subsequently, as an addendum.

Table 3. Effect of Avemar on the mutagenity of urethane

<b>Treatment</b>	<b>Mutation/wing</b>	<b>Mutational frequency</b>	<b>Significance Kastenbaum-B. test</b>
0,024% urethane	103/40	2,58	
0,024% urethane + 2% Avemar	61/40	1,53	p<0,01

Table 4.. Avemar effect on mutagenity of formaldehyde

<b>Treatment</b>	<b>Mutation/wing</b>	<b>Mutational frequency</b>	<b>Significance Kastenbaum-B. test</b>
0,042% formaldehyde + 10% Avemar	113/40	2,83	
0,042% formaldehyde + 10% sacharose*	43/40	1,1	p< 0,01
untreated control	34/40	0,85	

\* We added the 10% sacharose to the finished standard nutrient (already containing sacharose) subsequently, as an addendum.

### ***Evaluation***

1. Avemar is not mutagenic in the Drosophila somatic mutation and recombination test.
2. Avemar significantly decreased the mutagenity of all three examined toxicants.

### 3. Toxicology evaluation of Avemar in *Drosophila*

We observed in the experiments already performed that the Avemar supports the reproduction of flies. For the better determination we did two experiments.

*In the first experiment* the animals were kept on three different nutrients for two days, then the adults were removed, and on the 12<sup>th</sup> day we counted the hatching descendants. For the negative control we used a standard nutrient, the positive control consisted of yeast placed on standard nutrient. The living yeast is used world-wide for increasing the *Drosophila* reproduction. We made a thick liquid from the Avemar and the yeast and put one drop on the nutrient. The parental groups featured eight males and ten females of 15-17 days taken from the Oregon species 15 – 17. We performed the experiment at 25°C. The results are contained in Table 5.

Table 5. Effect of Avemar on *Drosophila* reproduction I.

<b>Treatment</b>	<b>Avemar</b>	<b>yeast</b>	<b>control</b>
No. of descendants	150	111	71

*In the second experiment* we used a nutrient containing 10% Avemar, the control group got a nutrient containing 10% sacharose. 8 males and 8 females from the Oregon species were kept on this for 5 hours then we placed them on standard nutrient for a period of 19 hours. Next day we placed the same animals onto a nutrient containing 10% Avemar and 10% extra sacharose for 5 hours then for 19 hours onto standard nutrient. After the 19<sup>th</sup> hour we removed the parental generation. We counted the hatching descendants on the 14<sup>th</sup> day. The experiment was carried out at 25°C. The results are contained in Table 6.

Table 6. Avemar effect on the *Drosophila* reproduction II.

<b>Treatment</b>	<b>no. of descendant</b>	<b>Treatment</b>	<b>no. of desc.</b>
0-5 hours 10% Avermar	3	0-5 hours 10% sacharose*	1
5-24 hours -	54	5-24 hours -	39
24-29 hours 10% Avermar	7	24-29 hours 10% sacharose	4
29-48 hours -	86	29-48 hours -	35
total descendants	150		79

\* We added the 10% sacharose to the finished standard nutrient (already containing sacharose) subsequently, as an addendum.

### **Evaluation**

The fermented wheat germ extract, Avermar has shown no toxicity in *Drosophila* at all. Avermar supported the reproduction of *Drosophila*, and could therefore be considered as an optimal biological value nutrient for these – nutritionally very vulnerable – animals.

Budapest, 2003-2004.

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