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## AIMS

Mice and rats are the most frequently used animals in experimental protocols performed in the European Union. They have several advantages when compared with other animals, such as their small size, and well-known anatomy, physiology, biochemistry and genetic. This work aimed to define the most adequate humane endpoints to recognize the pain in a rat model of mammary cancer chemically-induced.

## MATERIAL AND METHODS

Female Sprague-Dawley rat of four weeks of age were obtained from Harlan Interfauna. Animals from control group received only the vehicle (saline solution) (Figure 1). A list of biological parameters to be evaluated during the experiment was elaborated prior to the study and a score from 0 to 3 was attributed for each parameter. Severe alteration in some of these parameters were considered indicators of animal sacrifice (Figure 2). The animals were observed twice a day by the same researchers, for 18 weeks.

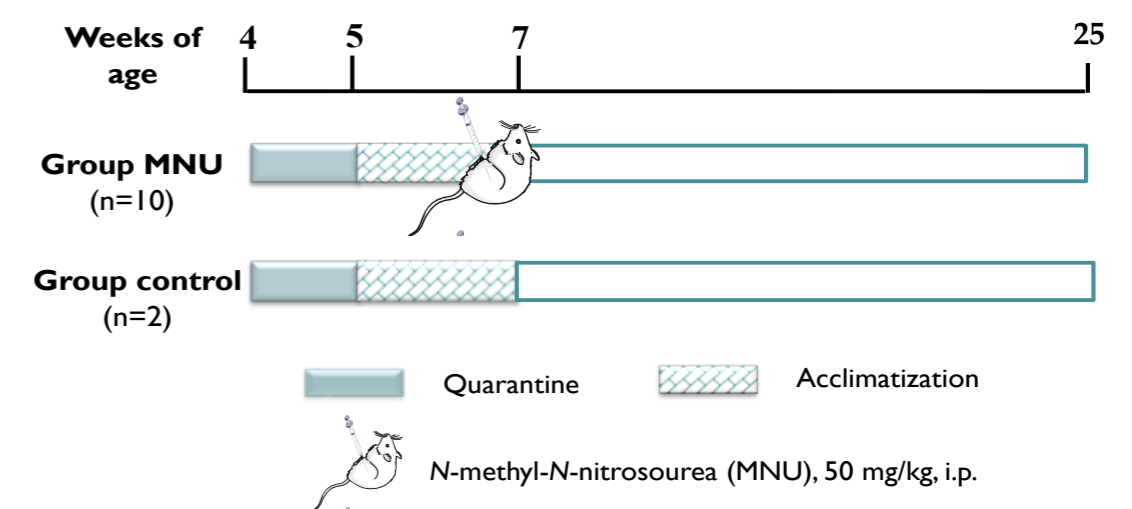


Figure 1. Experimental protocol.

<p><b>Body condition</b></p> <ul style="list-style-type: none"> <li>-1: overconditioned</li> <li>0: well conditioned</li> <li>1: under conditioned</li> <li>2: emaciated</li> </ul>	<p><b>Body weight</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: weight loss &lt;10%</li> <li>2: weight loss 10-20%</li> <li>3: weight loss &gt;20% *</li> </ul>	<p><b>Posture</b></p> <ul style="list-style-type: none"> <li>0: normal position</li> <li>1: changed position</li> </ul>	<p><b>Eyes and ears</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: partial closed eyes, droopy ears</li> <li>2: complete closed eyes, dropped and curved ears</li> </ul>
<p><b>Body temperature</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: hyperthermia</li> <li>2: hypothermia</li> </ul>	<p><b>Heart rate</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: increased</li> <li>2: decreased</li> </ul>	<p><b>Respiratory rate</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: increased</li> <li>2: decreased</li> <li>3: abdominal breathing</li> </ul>	<p><b>Location</b></p> <ul style="list-style-type: none"> <li>0: do not interfere with bodily functions</li> <li>1: interfere with bodily functions *</li> <li>2: in contact with cage floor *</li> </ul>
<p><b>Mental status</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: lethargic</li> <li>2: stupor</li> <li>3: moribund/coma *</li> </ul>	<p><b>Hydration status</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: abnormal skin pinch test</li> </ul>	<p><b>Mucosal</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: mild anemic</li> <li>2: moderate anemic</li> <li>3: severe anemic *</li> </ul>	<p><b>Mammary tumors</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: infected or ulcerated</li> <li>2: invasion of surrounding tissues</li> <li>3: persistent self-induced trauma</li> </ul>
<p><b>Response to external stimuli</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: mild</li> <li>2: moderate with vocalization</li> <li>3: violent</li> </ul>	<p><b>Coat and grooming</b></p> <ul style="list-style-type: none"> <li>0: normal</li> <li>1: lack of grooming</li> <li>2: rough coat, chromodachryorrhea</li> <li>3: piloerection and severe chromodachryorrhea</li> </ul>	<p><b>Burden</b></p> <ul style="list-style-type: none"> <li>0: &lt;35 mm in a 250 g rat</li> <li>1: &gt;35 mm in a 250 g rat *</li> </ul>	

Figure 2. Biological parameters evaluated weekly, for 18 weeks. The alteration in parameters marked with asterisk was indicator for animals' sacrifice.

## RESULTS

Six out 10 animals (60%) MNU-exposed developed a total of 21 mammary tumors (3.5 tumors/animal). Moderate anemic mucosal (score 2) were observed in one animal from group MNU at the 11<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> week of the experiment. No interference of mammary tumors with animals' bodily functions or alterations in mammary tumor surface that implied animal sacrifice was observed. At the end of the study, five animals (83.3%) developed at least one mammary tumor > 35 mm (Figures 3 and 4).

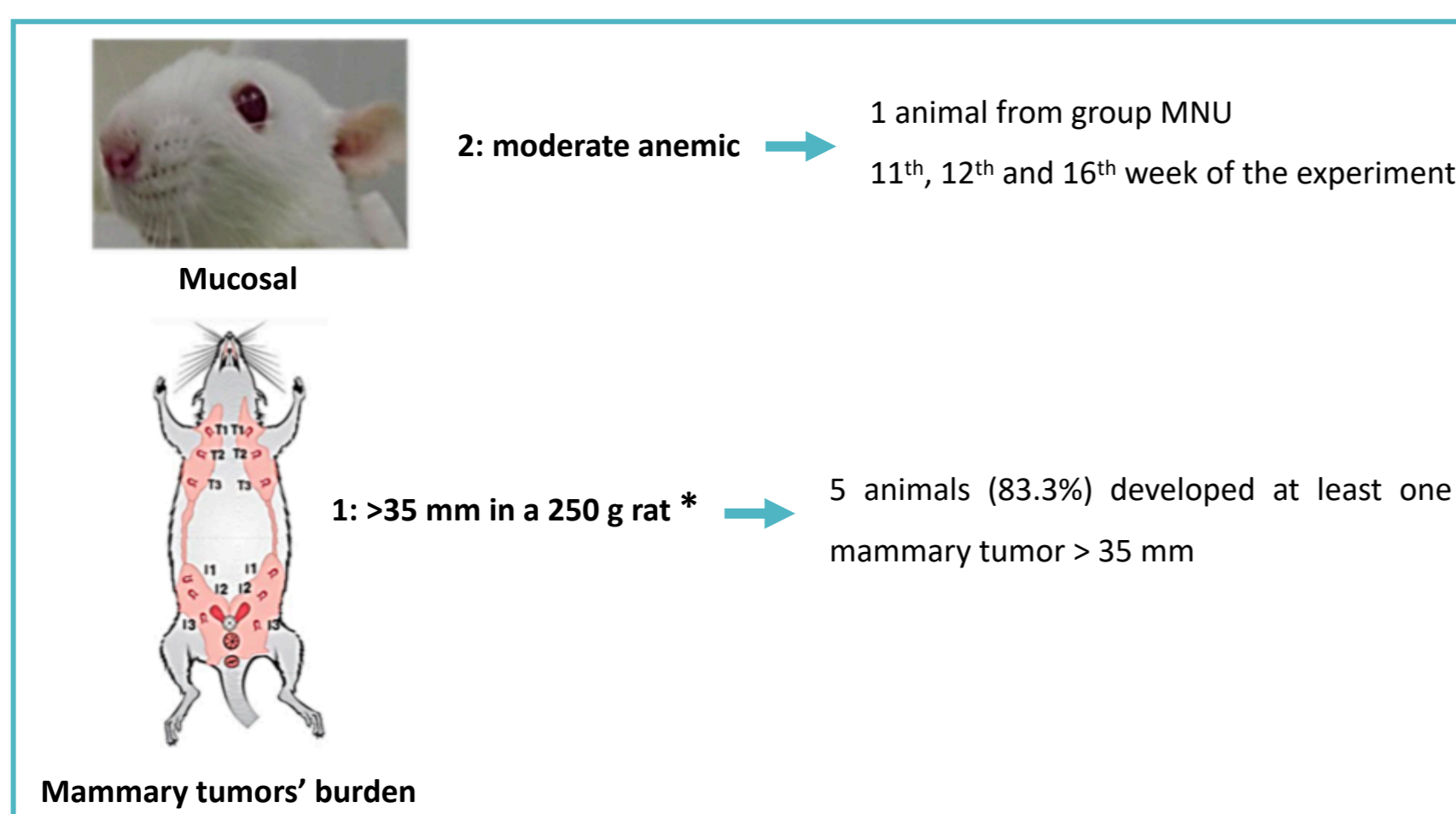


Figure 3. Alterations in the endpoints registered along the experimental protocol.

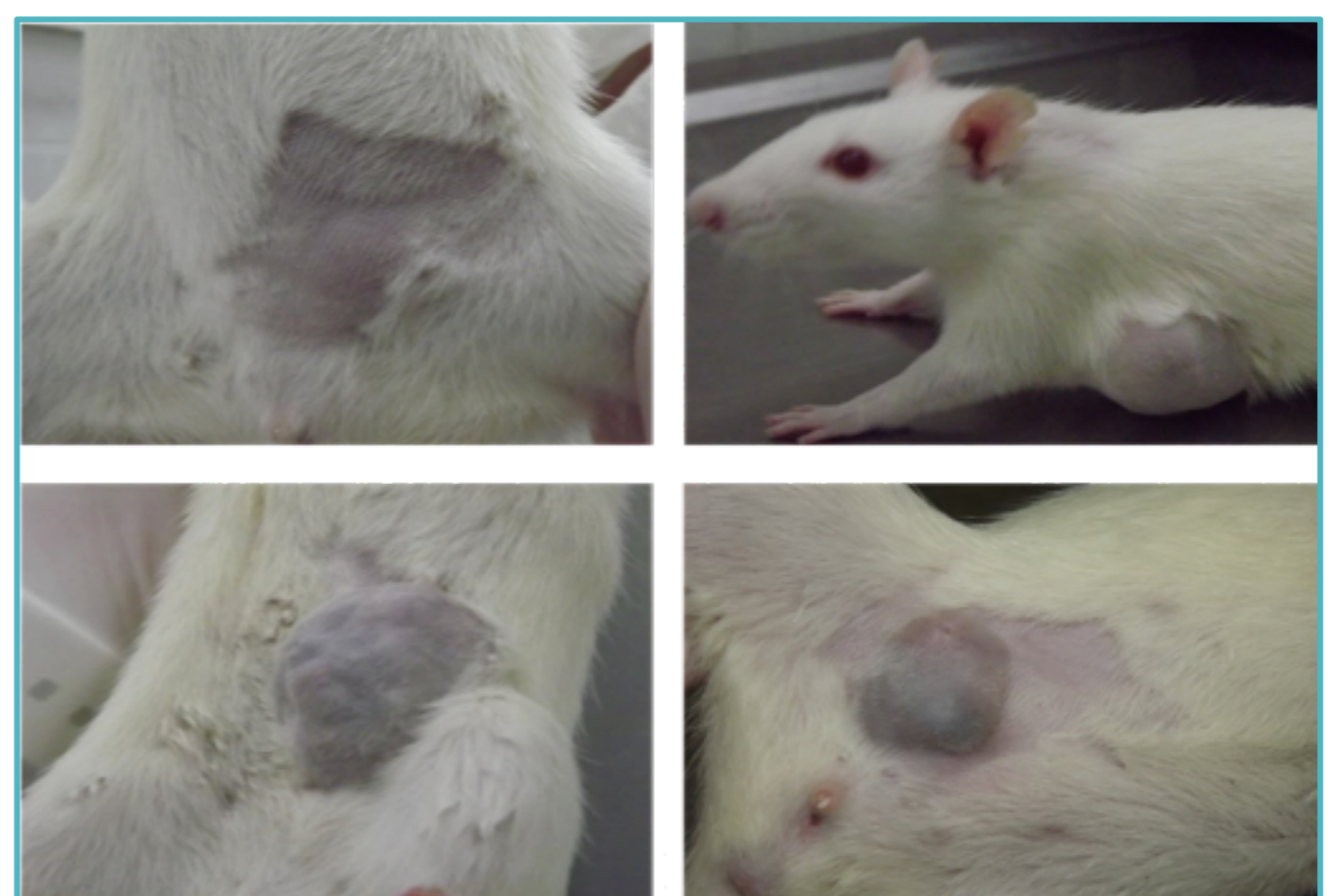


Figure 4. Six animals (60%) from group MNU developed 21 mammary tumors with different dimensions (3.5 tumors/animal).

**CONCLUSIONS:** We concluded that the alteration in only tumor dimensions does not imply the animals' sacrifice. The endpoints should be evaluated together, in order to define the most adequate time for humane animals' sacrifice.