CHRONIC EXPOSURE TO GLYPHOSATE AND 2,4-D INCREASES PULMONARY MELANOMA METASTASIS AND ALTERS HEALTH PARAMETERS IN C57BL/6 MICE

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INTRODUCTION: Glyphosate and 2,4-dichlorophenoxyacetic acid (2,4-D) are among the most widely used herbicides globally, ranking first and second in usage in Brazil. Due to their extensive use, both are frequently detected in drinking water and have been simultaneously identified in human biological fluids. AIM: Considering the Brazilian population chronic exposure, this study evaluated the individual and combined effects of glyphosate and 2,4-D on pulmonary melanoma colonization and on different health parameters in C57BL/6 mice. MATERIAL AND METHODS: Eight-week-old male mice were chronically exposed for 92 days to human-equivalent doses—adjusted for allometric scaling—of glyphosate (6.15 μg mL^{-1}), 2,4-D (0.369 μg mL^{-1}), or their mixture via filtered drinking water. A control group received only filtered water. Subsequently, 1.85×10^5 B16-F10 melanoma cells were inoculated into the caudal vein, and the exposure was maintained for 25 days. Water solutions were replaced twice weekly, and body weight, food intake, and water consumption were monitored throughout the experiment. At the end of the experiment, animals were anesthetized (10 $mg.kg^{-1}$ xylazine and 100 $mg.kg^{-1}$ ketamine) for blood collection (cell count and biochemical analyses), followed by euthanasia via cervical dislocation. Lungs, liver, spleen, kidneys, brain, heart, and testis were harvested and weighed for somatic assessment. The lungs, as the primary site of tumor implantation, were photographed to determine the area of colonized metastasis. RESULTS AND CONCLUSION: Animals exposed to herbicides exhibited increased food intake and reduced water consumption, which correlated with higher body weight, particularly prior to melanoma cells inoculation. Mice exposed to glyphosate and the mixture presented significantly larger lung metastases area ($p < 0.05$ and $p < 0.001$, respectively). While the somatic indexes did not significantly differ, the exposed groups presented notably heavier spleens. Although the differences in hematological and

biochemical parameters did not reach statistical significance, there were marked increases in cholesterol levels, alkaline phosphatase (ALP) activity, and white blood cell counts. Therefore, chronic exposure to glyphosate and 2,4-D, even at concentrations considered legally safe in Brazil, adversely affect physiological parameters and promoted metastatic progression in a murine model. These results raise concerns regarding the potential health risks associated with environmental exposure to these herbicides in human populations.

Keywords: Herbicides; B16-F10 cell line; melanoma; Brazil.

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